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CONTENTS

The Symposium on Probable or Possible Malignant Cervical Lesions will be continued in the November-December issue

Forthcoming Topics:

Inspection Technics in Carcinoma *in Situ*
Exfoliative Cytology and Experimental Cytology of Carcinoma *in Situ*
Clinical Viewpoints on Carcinoma *in Situ*
Morphogenesis and Incidence of Carcinoma *in Situ*

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Editorial

The Coming of Age of Cytology

Microscopic examination of cells for purposes of cancer diagnosis had been sporadically suggested as far back as the middle of the nineteenth century. In isolated instances and institutions the possibilities of this method of diagnosis were recognized relatively early. Thus, at Memorial Hospital in New York City, smear diagnosis of tumors has been extensively used since the year 1925 with a great deal of success and accuracy.[2, 3]

A world-wide acceptance of cytologic methods of diagnosis is relatively recent and inextricably associated with the name of Papanicolaou. Papanicolaou's meticulous work, based on solid clinical and histologic evidence, came forth at a time when cancer had become a major public health dilemma and at a time of increasing awareness that the cure of cancer is generally more secure and the prognosis more favorable when cancer is small and apparently in the early stages of development. Furthermore, Papanicolaou's initial effort was directed at the female genital tract, an area where the histologic bases of diagnosis of early cancer were firmly established during the first three decenniums of the twentieth century. It seems appropriate to mention in this context the names of Schauenstein, Pronai, Rubin, Schottländer and Kermauner, Schiller and Robert Meyer since they helped lay the histologic foundations by which cytologic diagnosis of cervix cancer was later built.

Despite initial incredulity and opposition, despite objections chiefly emanating from people without proper and adequate personal experience, cytology is coming of age. There can be no doubt in the mind of any serious student of this important addition to the medical armamentarium, that the diagnosis of cancer based on the strength of cellular evidence is accurate and reliable. Moreover, there are numerous practical applications of cytology in the fields of endocrinology, radiation treatment, enzyme studies, tissue culture, study of behavior of cancer cells in blood, and other areas of clinical and research medicine.

The foremost application of cytology and the one of most direct benefit to humanity is in the field of diagnosis of pre-clinical cancer: the search for early cancers of the cervix may result sooner or later in coming to terms with this dreaded disease. Moreover, cytology has also significantly contributed to the diagnosis of pre-clinical cancers of the lung,[8] the stomach,[6] the urinary bladder,[4] the buccal mucosa,[6] and the endometrium.[1] *In situ* carcinoma of many organs—a rare and accidental finding some years ago (except for the skin)—is rapidly becoming a household word in cancerology. For the benefit of those who still may have doubts as to the role of *in situ* carcinoma in the genesis of cancer it may be proper to quote Dr. Fred W. Stewart who once said: "Every infiltrative cervix cancer must come from *in situ* cancer, there being no other thing it can come from, this irrespective of various doubts cast on this relationship."[7] One may indulge, perhaps, in the exciting vision of more and more human cancers of various organs diagnosed in a curable stage by cytology. This vision is limited by lack of proper laboratory facilities, the staggering costs of large scale investigations

of this type, and, last but not least, by shortage of truly skilled professional personnel.

While a great many people are interested in cytology today, the number of qualified physicians with sufficient skills to render accurate cytologic diagnoses and to supervise the research effort that goes with it is almost despairingly inadequate. Diagnostic cytology is a difficult and demanding discipline and must be based on solid knowledge of histologic patterns of disease. The principles of microscopic diagnosis of cancer cannot be mastered during a short course, nor are they, like colposcopy, an appendage to the clinical examination of a patient. Diagnostic cytology is a branch of anatomic pathology and should not and cannot be practiced effectively at any distance from the autopsy room, the tissue laboratory, or the histologic preparation. This is a *sine qua non* for training of future generations of cytopathologists.

It is also appropriate to emphasize the necessity for proper channels of communication between the pathologist and the clinician in the field of cytologic diagnosis. Diagnosing smears by class only, no matter how useful in the past, constitutes a source of confusion today, largely because classes have not the same meaning to various people. Almost daily the writer finds on his desk smears without evidence of cancer, sent in for consultation purposes by a frantic physician because the label "class III" was affixed by the original examiner. This same examiner would have thought twice before spelling out: "suspect cancer in this material." In too many instances, in the writer's experience, the classes have become the repository of ignorance and the shield of the weak. The cytologic diagnosis must be rendered in clear terms of anatomic pathology with the same degree of reliability and accuracy as the diagnosis based on histologic evidence. Assuredly, there are situations where the evidence available is insufficient or the disease pattern precludes a definitive diagnosis. This fact may be simply communicated to the attending physician: if he is at all acquainted with the problems of microscopic diagnosis in general, he will readily comprehend this predicament.

Cytology does not belong in the hands of dilettantes but must be practiced by reliable craftsmen; if it is coming of age today, it still needs a great deal of tender professional care to reach widespread maturity.

LEOPOLD G. KOSS, M.D.

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A Cyto-Pathologic Study of Potentially Carcinogenic Properties of Air Pollutants

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SEVERAL reports in the literature indicate that air-borne compounds may contain carcinogenic agents;[6, 1, 3] thus, experimental carcinoma of the skin of mice has been produced by painting with the benzene solution of air pollutants.[7] Subcutaneous sarcoma resulted from the treatment of mice with tars extracted from atmospheric dusts,[8] and primary tumors of the lung developed in mice exposed to dust obtained from tarred roads.[2]

It was felt that additional confirmation of these reports was desirable. Also, a comparative quantitative evaluation of relative carcinogenic properties of air pollutants and tobacco tar may be of special interest from the standpoint of pathogenesis of bronchogenic carcinoma. Because of these considerations, the present investigation was undertaken, and the uterine cervix of mice, previously used in tobacco tar studies[4] was utilized again as a target organ. Cytologic and histologic evaluation of vaginal smears and uterine tissues were employed respectively for a more complete evaluation of the results.

Material and Methods

Animals. Two strains of mice, C3H and ZBC, were used in this study. C3H mice were from authors' colony, which was originated three years ago from breeders of Roscoe Jackson Memorial Laboratory, Bar Harbor, Maine. ZBC mice were obtained from Dr. John Bittner of the University of Minnesota.

Technic of Application of Tested Substances. Tested substances were applied to the cervix by previously reported technic.[4, 5] Details of intravaginal application of potential carcinogens are illustrated in Figure 1, which shows a mouse held on its back; the infant-size speculum affixed to the stand is inserted intravaginally. The lightbeam from the microscope lamp is directed with a head mirror through the speculum on the surface of the cervix where the tested substance is deposited by means of a wire-loop applicator. Intravaginal applications were started in virgin mice, six to eight weeks old and continued twice or five times per week, according to the experiment.

Types of Tested Substances. *1. Air Pollutants.* The air pollutants were collected on a glass fibre filter by the mechanical sampler illustrated in Figure 2. The sampler was installed on the fifth floor of the Hahnemann Medical College. This was arranged through the courtesy of the Department of Public Health of the City of Philadelphia, with a generous cooperation of Dr. Frederick Weber, to whom the authors acknowledge their indebtedness. Filters were changed every 24 hours. The total amount of material collected in 24 hours ranged from 150 to 480 mg. and averaged 298 mg. at the rate of about 47 cubic feet per minute of air forced through the

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Accepted February 1, 1961.

FIG. 1. Method of intravaginal application of the tested substances.

STRAIN / TREATMENT	C3H	ZBC
AIR POLLUTANTS	10	30
TOBACCO TAR	16	20
BENZOPYRENE	30	10
CONTROL	10	10
TOTAL	66	70

CHART 1. Distribution of animals according to the strain and the type of treatment.

filter. Only the organic fractions of air pollutants were used in this study. These fractions were separated with benzene in the Soxhlet Extractor. After the fractionation, benzene was completely removed by evaporation and the unaltered black, oily residue was used for intravaginal application five times a week throughout the entire experiment, *i.e.*, up to 80 weeks. According to the analyses routinely performed by the Government Air Pollution Control Agency, the organic fractions constituted about 50 per cent of the total material collected.[9] It was calculated that the amount used for each intravaginal painting was about 1 mg. Since the amount of air pollutants collected by a single sampler was insufficient for this study, additional filters from samplers operated by the Department of Public Health of the City of Philadelphia, and established in different parts of the city, were also utilized.

FIG. 2. Mechanical sampler used for collecting the air pollutants.

2. Tobacco Tar. The tobacco tar was obtained through the courtesy of Dr. Ernest Wynder of the Sloan-Kettering Institute for Cancer Research in New York, and was applied in its unchanged form five times a week until the termination of the experiment, that is, up to 92 weeks. The amount used for each painting was about .15 mg.

3. Benzopyrene. This was applied as a 1 per cent solution in acetone twice a week for 20 weeks, then the ZBC mice were sacrificed and C3H mice were left for observation for an additional eight weeks.

Follow Up Methods

Cytologic Evaluation. The vaginal smears were prepared every four weeks, stained and examined for the presence of eleven arbitrary cytologic criteria of malignancy, routinely employed by authors in the cellular evaluation of the development of the cervical carcinoma.[4] These criteria are: nuclear

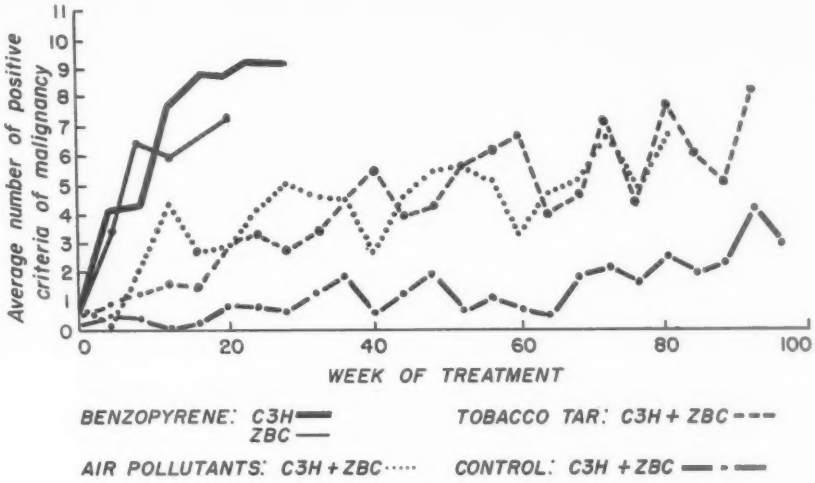

CHART 2. Average number of positive cytologic criteria of malignancy calculated per mouse in each group every four weeks.

HISTOLOGICAL CLASSIFICATION / LENGTH OF TREATMENT	NO TISSUE OR POOR TISSUE	NORMAL TISSUE	BASAL CELL HYPERPLASIA AND/OR DYSPLASIA	CA. IN SITU	CA. IN SITU WITH MICROINVASION	EARLY INVASION SQU. CELL CA.	INVASION SQU. CELL CA.
0–20 WEEKS	◆○○○○ ○●●●△ ▲▲▲▲▲		△		◇◇		◆◆◆◆
20–40 WEEKS			◇◇●	◇◇	◇◇◇	◆◆◆◆◆ ◆◆◆◇	◆◆◆◆◆ ◆◆◆◆◆ ◆◆◆ ◆◆
40–60 WEEKS		△△△	○○				○
60–80 WEEKS		○●	○○○○○ ○○○●△ △△△△△ △	△▲▲	●	○●	
80–100 WEEKS		○	○○○○○ ●△△△△ ▲▲▲▲	●△△△△ ▲▲▲▲	○○○○▲ ▲	○○△▲▲	

○ △ ◇ *Indicate ZBC strain*
● ▲ ◆ *Indicate C3H strain*
◐ *Air pollutants*
◭ *Tobacco tar*
◇ *Benzopyrene*

CHART 3. Histologic diagnoses in each strain and group presented in relation to the length of time of treatment and the lifespan.

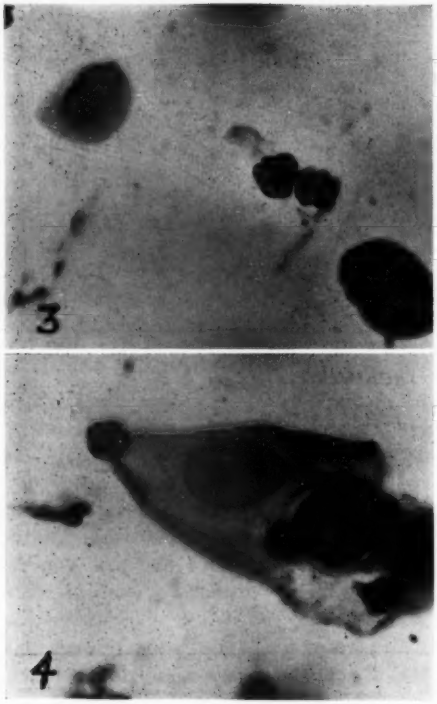

FIG. 3. Normal cells found in a vaginal smear of an untreated ZBC control mouse after 76 weeks of observation. FIG. 4. Atypical cluster of cells displaying keratinization and engulfment in the smear of a C3H mouse treated with benzopyrene for 16 weeks.

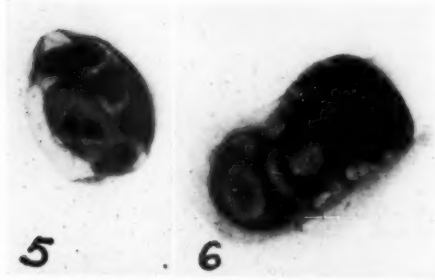

FIG. 5. Keratinization and engulfment, as in Figure 4, but found in the smear of a ZBC mouse treated with air pollutants for 48 weeks. FIG. 6. A localized area of cytoplasmic basophilia in a smear of a benzopyrene-treated C3H mouse, two weeks after the completion of a 20-week course of treatment.

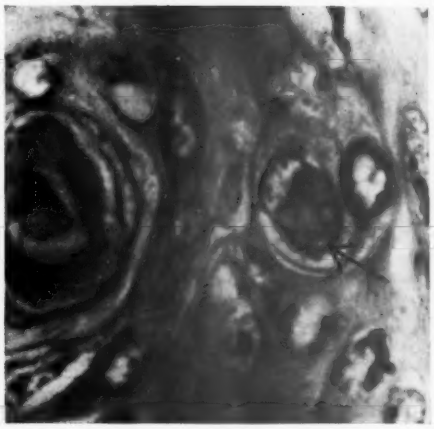

FIG. 7. Basophilic cytoplasmic areas as in Figure 6, found in the tissue of the same mouse and photographed at the same magnification.

enlargement, hyperchromasia, irregular nuclear borders, prominent nucleoli, bi- and multi-nucleation, localized areas of cytoplasmic basophilia, cytoplasmic vacuolation, formation of bizarre clusters, presence of elongated cells, engulfment and keratinization.

Histologic Diagnoses. At the time of autopsy the uteri were grossly examined, fixed *in toto* in 10 per cent formalin and cut antero-posteriorly. Sections were made from the right and left halves of each specimen.

Experimental. Among mice of each of the two strains used, there were three groups of treated animals: those treated by air pollutants, tobacco tar and benzopyrene respectively. The fourth group consisted of untreated controls. The distribution of animals per group and per strain is shown in Chart 1.

Upon the completion of experiment, the following results were obtained.

Results

Cytologic Findings. In general, there was no substantial difference between the cytologic findings in smears of C3H and ZBC

mice. Therefore, evaluation of cytologic findings in groups of mice of these two strains is considered together, whenever they were subjected to the same treatment, except in case of mice treated with benzopyrene, where each strain responded to the treatment differently. Typical cellular findings are illustrated in Figures 3-6, 8-12.

Chart 2 illustrates in graphic form, the comparative development of cytologic criteria of malignancy according to the type of treatment. The average number of positive cytologic criteria of malignancy calculated per mouse in each group are marked on the ordinate and are plotted against the length of time of the treatment, marked on the abscissa, in four-week periods. The results of previously reported experiments[4, 5] indicated that the persistence of at least five

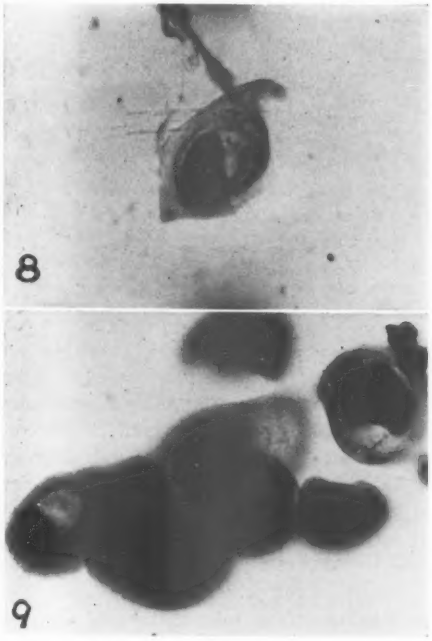

FIG. 8. Cells with basophilic areas in the cytoplasm observed in the smear of a ZBC mouse treated with air pollutants for 12 weeks. FIG. 9. Localized areas of cytoplasmic basophilia encountered in a C3H mouse treated with tobacco tar for 78 weeks.

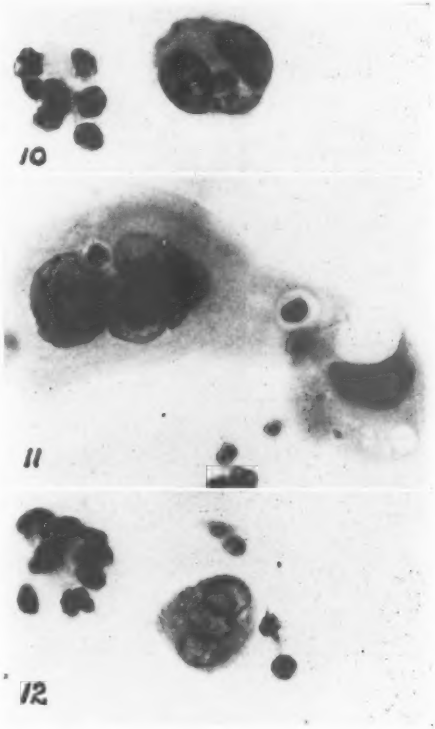

FIG. 10. Multinucleation was observed in a vaginal smear of a benzopyrene-treated C3H mouse. FIG. 11. Multinucleation was also seen in a smear obtained from a ZBC mouse treated with air pollutants for 80 weeks. FIG. 12. Multinucleation appeared likewise in a smear of a C3H mouse treated with tobacco tar for 78 weeks.

cytologic criteria suggests presence of a malignant neoplasm. This chart shows that the application of benzopyrene resulted in a rapid meeting of a steadily increasing number of these criteria. In animals treated with the air pollutants and tobacco tar, cytologic findings suggested presence of early malignant neoplasm after a very prolonged treatment. The upward tendency was interrupted by many oscillations. The untreated control mice exhibited some cellular abnormalities, the appearance of which was more frequent as they grew older.

Histologic Findings. Results of the histo-

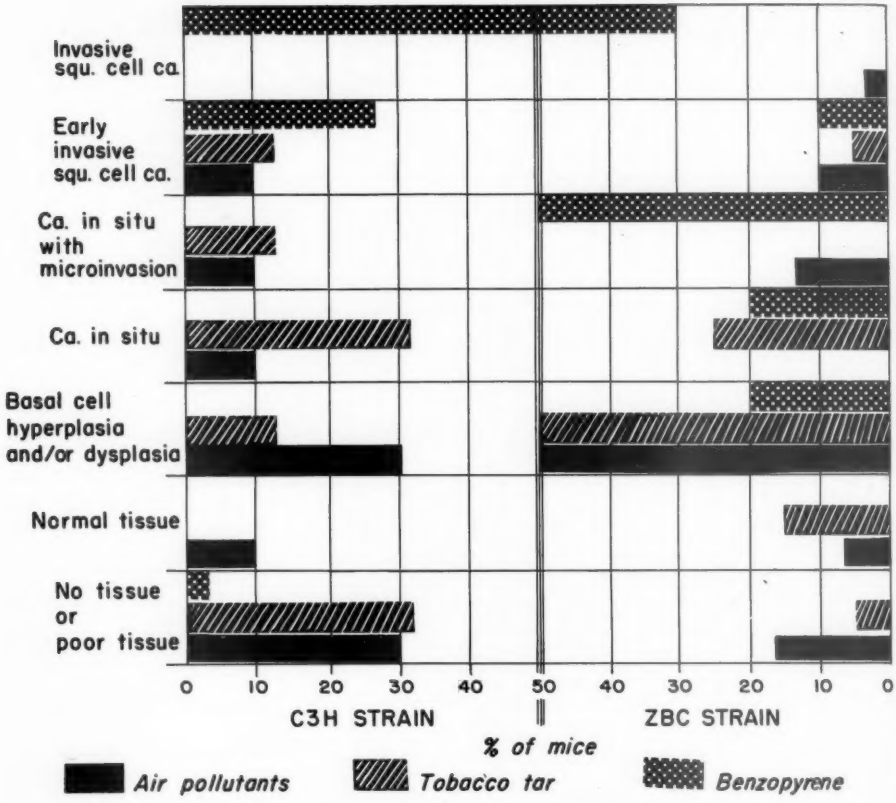

CHART 4. Percentage of mice with different histologic lesions, according to the strain and the type of treatment.

logic examination of lesions, produced in each group of each strain, and the treatment are presented in Charts 3 and 4. In Chart 3, diagnoses are tabulated according to the length of time of treatment. Six different types of histologic lesions, arranged in order of progressive anaplastic changes, are plotted against the 20-week periods of carcinogenic applications, to show the relation of the type of histologic lesion to the length of treatment. The control mice are excluded from this chart, their histology being, in general, within the "normal tissue" group, except for a single ZBC mouse which displayed a rather pronounced basal cell hyperactivity. Chart 4 shows the percentage of mice of each strain, according to the type of treatment, exhibiting the various types of histologic lesions considered in the former chart. Controls are omitted. Figures 13-25 illustrate examples of different types of histologic lesions produced.

Discussion

Results of the present study indicate that such substances as tobacco tar and air pollutants may produce squamous cell carcinoma in mucous membranes of mice. Such cancers appear later in the life of a certain proportion of animals after pro-

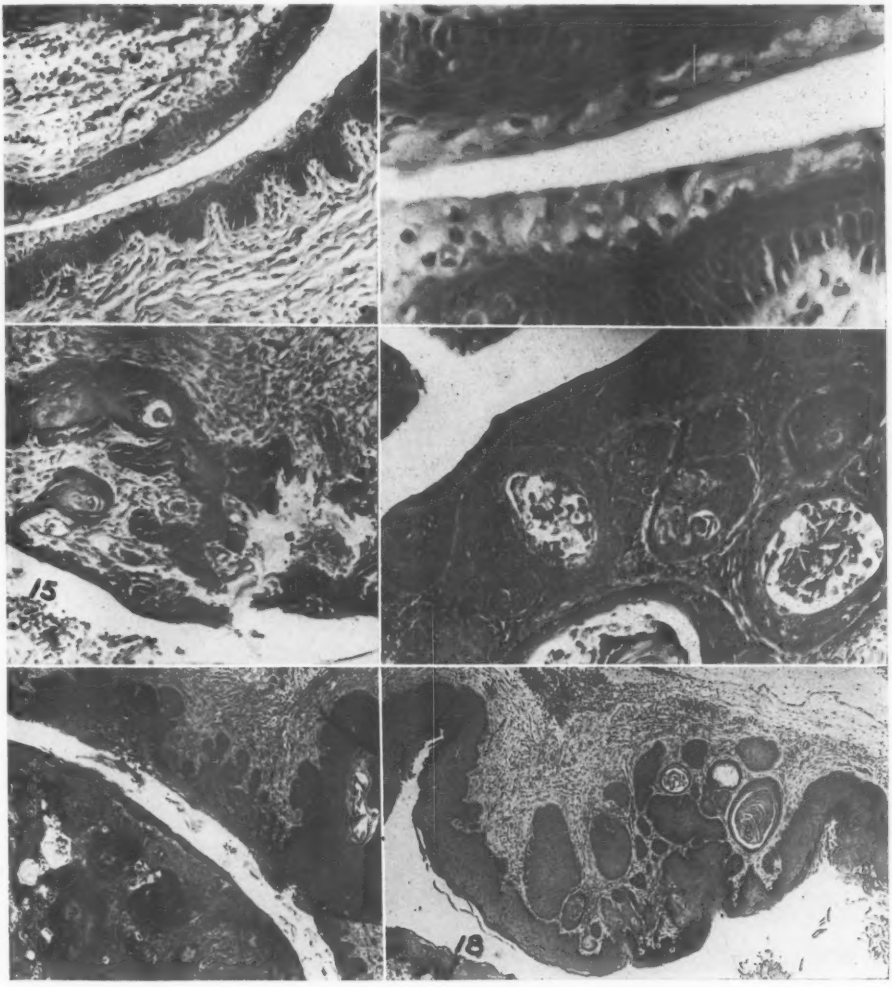

FIG. 13. Normal tissue from the cervix and vagina of a control untreated ZBC mouse, observed for 95 weeks, magnified 125x. FIG. 14. The same field as in Figure 13 but magnified 500x, shows a normal diestrus with a covering layer of mucoid cells in the cervical epithelium facing vagina with some basal cell hyperplasia underneath covering mucoid cells. FIG. 15. An early squamous cell carcinoma, cervix, produced in a C3H mouse by benzopyrene, magnified 125x. FIG. 16. A more advanced squamous cell carcinoma of the cervix produced in another C3H mouse by benzopyrene eight weeks after completion of 20 weeks of treatment, magnified 125x. FIG. 17. A low power view (50x) shows an advanced squamous cell carcinoma, cervix, facing an early invasive and an adjacent in situ carcinoma of the vagina in another C3H benzopyrene-treated mouse. In tissues of the same mouse, localized areas of cytoplasmic basophilia within the early invasive carcinoma were found (See Fig. 7). FIG. 18. Early invasive squamous cell carcinoma produced in a ZBC mouse treated with air pollutants (50x).

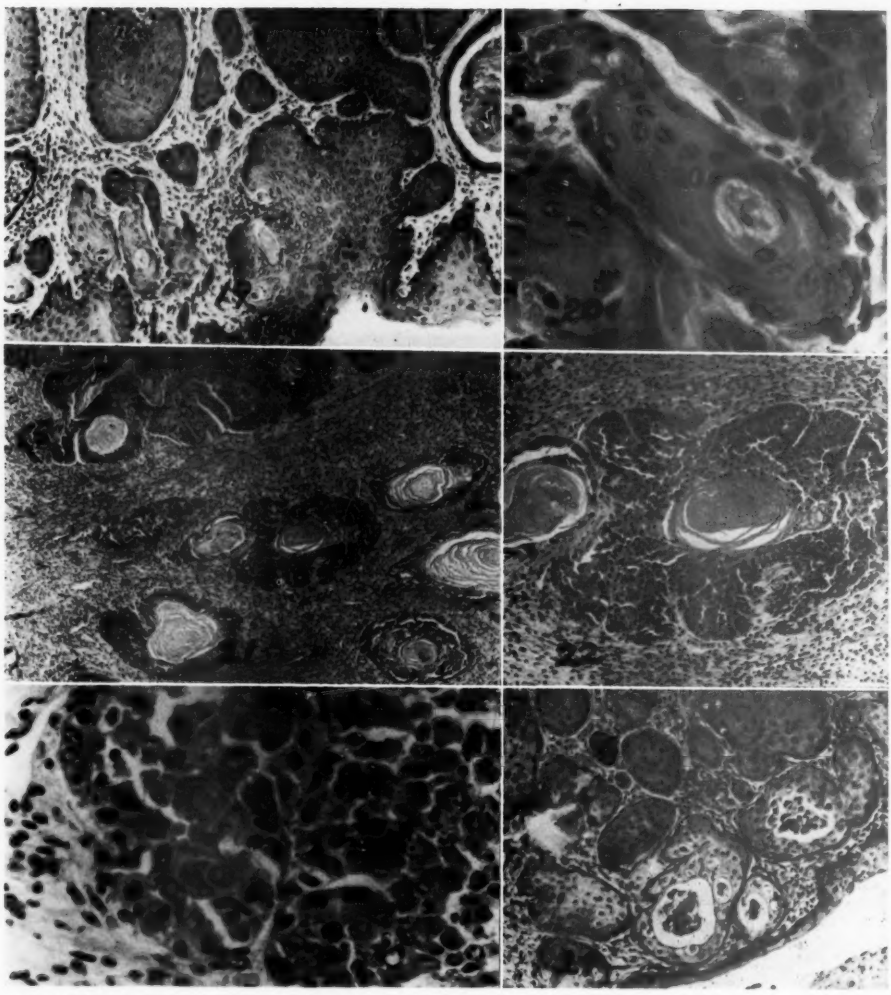

FIG. 19. The same as in Figure 18 (125x). FIG. 20. The same as in Figure 19 (500x). FIG. 21. A keratinizing advanced carcinoma produced in only one ZBC mouse treated with air pollutants. The tumor was necrotic (50x). FIG. 22. The same as in Figure 21 (125x). FIG. 23. The same as in Figure 22 (500x). FIG. 24. Early invasive carcinoma induced in a C3H mouse by tobacco tar (125x).

longed and frequently repeated exposure.

In the series of animals herewith presented, the appearance of tobacco tar-induced neoplastic lesions was more sluggish than in a previously reported series.[5] Certain variations in their reaction to carcinogenic agents were observed by the authors among different batches of mice used in various experiments. It is of interest, however, that air pollutants investigated in the present batch of mice had at least as pronounced an effect as tobacco tar did in two different strains of mice.

Only one mouse died with a large, in-

vasive, necrotic, air pollutant-induced carcinoma, while all experimental mice died, or were sacrificed in extremis of benzopyrene-induced cervical carcinoma. It was clearly observed, however, that air pollution-induced morphologic lesions are indistinguishable from those encountered in different stages of development of benzopyrene-induced, indisputable cervical cancers.

For practical considerations, most mice do not live long enough to reach later stages of development of air pollution and tobacco tar-induced lesions. The existence of occasional progression was clearly demonstrated in a small proportion of animals.

Summary

Comparative carcinogenic properties of air pollutants, tobacco tar and benzopyrene were tested by means of correlated cytopathologic studies, utilizing the uterine cervix of mice as a target organ. It was demonstrated that ZBC and C3H mice treated intravaginally by air pollutants and tobacco tar develop cellular abnormalities and histologic lesions, which are morphologically indistinguishable from those accompanying the development of benzopyrene-induced carcinoma. The ultimate stage of development is, in general, reached later and is less advanced than in benzopyrene-induced lesions, so that after 95 weeks of treatment, only a very small percentage of mice showed an early invasive carcinoma. The oscillating nature of cytologic findings, observed in a series of consecutive smears of air pollution and tobacco tar-treated mice contrasted with the steady progression of benzopyrene-induced cellular abnormalities, and may suggest the presence of successive, possibly multicentric mucosal lesions, some of which desquamate entirely before others progress to invasive carcinoma.

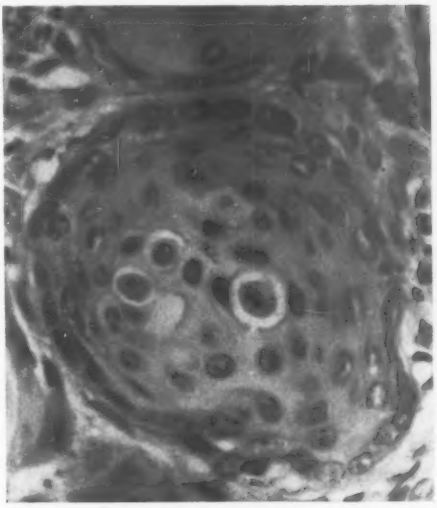

FIG. 25. Early invasive carcinoma induced in a C3H mouse by tobacco tar (500x).

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The Cytology of Pregnancy*

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Introduction

SINCE Papanicolaou in 1925 presented for the first time his vaginal cytology study of pregnant guinea pigs, many studies of the cytology during human pregnancy have been published. Most of these studies fall into two groups: those investigating malignant or premalignant changes occurring during pregnancy, and those aimed to correlate cytological changes with the course of pregnancy and its pathological physiology. Both have been the subject of recent symposia published in ACTA CYTOLOGICA. In the first symposium, entitled "Cancer Cytology During Pregnancy," 54 authors discussed the various aspects of epithelial dysplasia, carcinoma *in situ*, and invasive cancer as it occurs during pregnancy. It was generally agreed that cancer occurs in the average percentage of .02 per cent, or in one out of every 5,000 pregnancies, and that approximately 1.6 per cent of patients with cancer of the cervix were pregnant. There was also general agreement that the prognosis for cancer of the cervix is the same during the first trimester of pregnancy as in nonpregnant patients, while in the last trimester and in postpartum cases the prognosis is generally poor.[13] The important question, Should all pregnant women be screened for cervical carcinoma? has been answered in the affirmative by all participants of the symposium. In the second symposium on "Hormonal Cytology During Pregnancy and the Postpartum Period," 44 authors discuss the importance of vaginal cytology as a prognostic tool in pregnancy disorders, and it is this problem I wish to make the subject of my review.

Hormonal Factors During Pregnancy

We all are familiar with the tremendous hormonal changes which take place from almost the first day of pregnancy and which form the basis for the many pregnancy reactions or pregnancy tests in use today. If it is possible to use these hormonal changes to create a decisive response in experimental animals permitting the recognition of pregnancy with a high degree of accuracy, the question may rightly be asked, Why shouldn't it be possible to detect the same hormonal response on the epithelial cells of the pregnant woman? Jean de Brux[7] divides the factors which influence the epithelium and connective tissue during pregnancy into the maternal hormones (pituitary, ovary, and adrenal), the placental hormones, and the fetal hormones. According to Zander,[51] trophoblasts start to produce chorionic gonadotrophin a few days after implantation of the egg, and the production of steroids also starts during the first months of pregnancy. This implies that the pregnancy-preserving function of the corpus luteum beyond the first months of pregnancy has probably been overestimated. From experiments with isotope-marked progesterones and with measurement of the hormone difference in the arterial and venous vessels of the placenta, it has been estimated that at least 25 to 50 mg. of progesterone per day are produced during the first half of pregnancy and up to 280 mg. of progesterone during

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the second half of pregnancy. The excretion of estrogen during pregnancy also increases about 100 times as compared to the normal cycle, and Brown[6] found that the estrogens produced by the placenta increased from 1 mg. per day in the tenth week of pregnancy to 50 to 100 mg. per day at the end of pregnancy.

The epithelial modifications during pregnancy have long been known and consist of hyperplasia and squamous metaplasia of the epithelium with a curious phenomenon that during pregnancy "the squamous epithelium never advances completely to maturation; the intermediate cells, although in more numerous layers than normal, never become eosinophilic and karyopyknotic, and sometimes exfoliate in a characteristic form, the navicular cell."[7] It is needless to emphasize that navicular cells can also be seen in the vaginal smears of nonpregnant women and therefore cannot be considered specifically diagnostic of pregnancy. However, Papanicolaou[24] stresses the fact that during pregnancy they show certain distinguishing characteristics: larger nuclei, heavier cell outlines and more compact grouping.

Cytological Technic

The most important factor in the hormonal evaluation of cytological smears during pregnancy is the technic of preparation of the smears. Pundel[26] is quite adamant in demanding that vaginal smears be collected from the lateral wall of the vagina after insertion of a dry speculum, and he emphasizes that cervical scrapings or aspirations from the vaginal pool have no value for a hormonal cytodiagnosis during pregnancy. Wied[47] stresses the fact that the lower part of the vagina always shows increased cornification which will contaminate smears prepared without the use of a speculum. Peters[25] also stresses the difference in sensitivity to hormones of the vagina and the portio. Our own studies fully agree with these statements. After correct collection of the slide the Papanicolaou staining technic is recommended by most authors. Gaudefroy[11] prefers the hematoxylin Sudan III method of Shorr since it gives better and more reproducible results for the hormonal cytology of pregnancy. For proper evaluation of cells on a quantitative basis, the determination of the karyopyknotic or the eosinophilic index has been recommended. They are approximately parallel and show a sharp decline of the indices during the first three months with an increase in the last month of pregnancy. In our study we felt that differential counts or a determination of the karyopyknotic index did not alter appreciably the evaluation of the smear when it was performed by an experienced cytotechnologist.

Diagnosis of Pregnancy by Means of Cytology

The cytologic diagnosis of pregnancy has not been successful up to now.[15] A typical pregnancy smear can be observed generally only after three months, at which time a trained obstetrician can make the correct diagnosis by clinical examination.[27] In reviewing over 3,000 cases of early pregnancy and amenorrheas where pregnancy could be suspected, Pundel could only make a positive diagnosis of pregnancy in 72 per cent, while in 9 per cent a false-positive diagnosis was made. Using the cells from the urinary sediment, Meyer and von Haam[21] were able to diagnose only 68 per cent correctly as pregnant, and among our negative controls we had 11 per cent false-positives. In other words, 68 per cent of our cases had coinciding positive frog tests and navicular cells in the urine samples, 21 per cent had no navicular cells in the urine and the frog test was positive, and 11 per cent showed navicular cells in the urine and the frog test was negative. Oral smears are even less characteristic since they do not show any navicular cells.[40]

The administration of small doses of estrogens for three to five days previous to

repeating the vaginal smear has been recommended recently as a more accurate technic for the cytologic diagnosis of pregnancy. The nonpregnant woman will react to it with a marked estrogenic effect which will not appear if the woman is pregnant.[48] It has been shown that this negative response of the vagina after oral or parenteral administration can only be explained by the fact that these substances under such conditions act not as estrogens but as progesterone-like substances.

Normal Vaginal Cytology During Pregnancy

Pundel in his excellent monograph with Van Meensel[33] and his many subsequent papers studied exhaustively the changes which occur in normal pregnancy until the onset of labor. He speaks of three phases, the first of which reflects the hormonal activity of the corpus luteum, while the second and third phases reflect the hormonal activity of the placenta. Since characteristic cell changes appear only in smears without cytolysis, one has to separate for practical purposes cytolytic cytology from noncytolytic cytology. In the presence of conception the cell clusters of the menstrual smear do not disappear but persist in the absence of menstruation. The superficial cells progressively disappear so that the eosinophilic index goes below 5 and the karyopyknotic index below 10. The intermediate cells now tend to shape into the navicular cells and at the end of the third month the pregnancy type of vaginal smear is well established. It is exceptional, according to Pundel,[28] to see pregnancy navicular cells before the second week after the last menstruation. During this phase short-lasting rises in the karyopyknotic and/or eosinophilic index may occur up to the third month. They are transitory and may recur at monthly intervals, reflecting a persistent cyclic activity of the ovary during the first trimester of pregnancy. After the third month of pregnancy vaginal cytology takes on a uniform pattern.[44] Typical navicular cells appearing in thick clusters become a predominant element of the smear. The eosinophilic index and the karyopyknotic index remain low.

A certain percentage of vaginal smears are of the cytolytic type. This means that the majority of cells have lost their cytoplasm and demonstrate only the typical nucleus of the intermediate cells.[45] Accompanying this phenomenon is usually a pure or predominant growth of Döderlein bacillus. By applying local antibiotics these smears can be transformed into the usual navicular type of pregnancy smears.[41] At the present time the significance of the cytologic smears has not been agreed upon. We agree with Pundel[29] that they are compatible with normal pregnancies and have no prognostic significance for the prenatal diagnosis of the sex of the baby. Their occurrence interferes of course with the hormonal evaluation of the smears, and for this reason transformation into normal pregnancy smears is indicated.

Two other types of smears which may occur during pregnancy are the inflammatory type and the estrogenic type. In the inflammatory type, cervicitis is usually present to a more or less severe degree, with numerous leukocytes and a mixed bacterial flora. Mucus is present, and individuals usually profess to suffer from an increased vaginal discharge. The eosinophilic index is high due to pseudoacidophilia, and the cells show perinuclear vacuoles with pictures of karyolysis, karyorrhexis or dyskaryosis.[46] In addition to pseudoacidophilia, the plasma of these cells shows granules and invasion by leukocytes. In the estrogenic type of smear navicular cells are present in small numbers without characteristic clumping. The smear is dominated by the appearance of acidophilic and pyknotic superficial cells typical of preovulatory type of smears. In our study the

inflammatory type of smears occurred during the entire period of pregnancy and made up as much as 35 per cent of our material, while the estrogenic type of smear was found predominantly during the first six weeks of gestation and never exceeded 15 per cent of our material. Its persistence points to a serious hormonal disturbance of the pregnancy and is of considerable clinical importance.

The vaginal flora of the pregnant woman is only different in degree from that of the nonpregnant woman.[1] The study of Bret and co-workers[4] showed that two-thirds of all cases of vaginitis in a pregnant woman were caused by Staphylococci and Trichomonas. They found the incidence of vaginal infection much higher in pregnant women than in nonpregnant women, although the incidence of symptoms from vaginal infection appears lower in pregnant women than nonpregnant women.[14] Pundel[30] feels that Monilia can be found more frequently in pregnant women. Wied[49] explains the frequency with which Döderlein bacilli are found in such large numbers in the vaginal flora, causing the phenomenon of cytolysis, by the fact that the cellular maturation does not proceed to complete cornification although the epithelium proliferates at least to the intermediate cell layers. Pregnancy, of course, is a classical example in which this condition is encountered. This can be controlled effectively by a single intravaginal administration of an antibiotic which will inhibit the growth of Bacillus vaginalis and immediately stop cytolysis and the consequent leukorrhea.

Vaginal examination has been stressed recently as an important clinical test at the end of the pregnancy. Lichtfus[20] differentiates the pregnancy-at-term smear from the pregnancy-prior-to-term smear and the postpartum smear. The pregnancy-at-term smear appears in the last two weeks of pregnancy and is characterized by a diminution of the number of cell clusters without change in the shape and staining quality of the cells. In addition, the eosinophilic index and the karyopyknotic index rise and an increased number of isolated and flattened cells appear. In some cases this smear type is followed by the appearance of basal cells found in the postpartum cell smear. This smear type according to Pundel[31] indicates serious danger for the fetus either through true postmaturity or through placental senility. Similar changes are also described by Ramos[37] in urocytograms. In our study with Meyer[21] we could confirm the observation of Pundel with regard to the vaginal smears but found just the opposite condition in smears prepared from urinary sediment. Here large plaques of navicular cells appeared in the last week of pregnancy. We re-examined our material for the appearance of an antepartum cell stressed by Wied[50] and found it in 30 of 100 urines of men and nonpregnant women and in only 64 per cent of the urines of pregnant women.

The entire problem of the antepartum smear still needs further investigation to resolve the many divergent opinions as to its practical value. We agree with Pundel that hormonal evaluation cannot be accurate unless it is performed on a noncytolytic smear which has been taken from the lateral wall of the vagina with the use of a speculum in order to avoid cervical and vulvar contamination. It is also important that smears be taken at frequent, perhaps daily, intervals, that the technic always be the same, and that quantitative estimations such as the determination of the karyopyknotic and eosinophilic indices be carried out. Should the value of the antepartum smear be confirmed, we would agree with Pundel's enthusiasm as to its importance for modern obstetrics. He was able to show that by use of this smear unnecessary premature induction of labor could be avoided, and on the other hand artificial induction

of labor could save the child. We feel that this very important phase of vaginal cytology urgently needs further confirmation.

Once labor has started the rupture of the fetal membrane adds a new cell type to the vaginal smear—the vernix caseosa cells of the fetus. They can be identified by their polygonal shape, lack of nucleus, and the light yellowish stain. They must be differentiated from the anucleated squamous cells that occur after contamination of the vaginal smear with vulvar cells.[16]

The postpartum period can be divided into the immediate postpartum period lasting until the tenth day and the prolonged postpartum period lasting from the tenth day until the return of the menses in the sixth week for nonlactating women and up to four months for lactating women. The immediate postpartum period produces a vaginal smear resembling castration or menopause in many respects. Blood and numerous pus cells are present in the first days, which are followed quickly by large numbers of histiocytes. The navicular cells disappear quickly and numerous parabasal cells appear for which Papanicolaou[24] coined the term "postpartum cells." They are characterized by thick, prominent margins, well-formed, partly pyknotic nuclei, and a vacuolated bluish cytoplasm. In the second week after delivery the erythrocytes disappear and the postpartum cells assume more the configuration of normal parabasal cells. This can be explained by the decrease in glycogen in the cytoplasm.[23] From then on the smear in nonlactating women slowly assumes the normal preovulatory aspects with a steady increase of intermediate and superficial cells. In the lactating woman parabasal cells persist for a considerable time and the smear retains the aspect of the atrophic smear of the menopausal or castrated woman. An interesting dissociation between the behavior of the endometrium and the vagina in the postpartum period has been emphasized by Kamnitzer.[17] Peters stresses a difference in the sensitivity between the vaginal epithelium and the portio epithelium to the re-awakened hormonal activity of the ovary and recommends that smears from both regions should be taken into consideration. In the urocytogram Ramos describes oligocellular exfoliation with basal cells predominant, a tendency towards eosinophilia, abundant histiocytes, leukocytes and red blood cells.

No information is available concerning the appearance of decidual cells in the vaginal smear during normal pregnancy. It must therefore be presumed that they do not occur. In the cases of ectopic decidua, however, the cervical stroma quite frequently shows a decided decidual reaction which according to Novak[22] is of little clinical importance and has no influence upon the course of pregnancy. Decidual cells in the cervix have been recognized since 1885. Cervical deciduosis can lead to metrorrhagia during pregnancy, and according to Lepage and Schramm[19] can evoke cervical carcinoma. The cervical smear usually shows a normal hormonal equilibrium. Inflammation is frequent and cytolysis is often present. Decidual cells in the smear are described by Tasso and coworkers[43] as having the size of an external basal cell with grayish cytoplasm and an eccentric nucleus with finely dotted chromatin. They must be differentiated from external basal cells and navicular cells as well as from cytotrophoblasts, which is a tedious and time-consuming task. Fortunately cases of cervical deciduosis are not frequent and in the absence of a threatening abortion cytotrophoblasts do not enter the picture. Under the influence of ulceration or secondary infection decidual cells may show certain anomalies which make their recognition still more difficult, and the cyanophilic plasma of the cells becomes bloated or shrunken and the nuclei become almost pyknotic.[5]

Cytology of Abortion

Gaudefroy[12] states that the incidence of spontaneous abortion is approximately 15 per cent of pregnancies, and that 6 to 7 per cent of all pregnancies will abort due to endocrine disorders. He then poses the interesting questions: Is it possible to recognize by means of cytology abortions threatened by endocrine factors? What is the prognosis of endocrine pregnancy disorders? and what is the effect of administered hormone treatment? If such sources for diagnostic errors as vaginal douches, infections, etc. are carefully eliminated, he feels that the diagnostic accuracy of the cytohormonal prognosis is very high and therefore clinically useful.

Pundel[32] states that in his experience a normal pregnancy smear guarantees a normal delivery in over 90 per cent of the cases. Cytolytic smears, while rightfully counted among the normal pregnancy smears, may occasionally hide an abnormal hormonal condition, and for this reason he recommends for correct evaluation a transformation of a cytolytic smear by means of local antibiotics. Abnormal pregnancy smears have a high diagnostic and prognostic significance, according to this author. An eosinophilic index of over 50 in several smears taken at intervals of a few days predicts a very poor chance of saving the pregnancy once it is older than three months. A smear resembling the postpartum type with the majority of large parabasal cells persisting for longer than four days indicates the death of the fetus. Hormonal bioassays carried out simultaneously with the vaginal smears showed a complete agreement between the hormonal findings and the vaginal smears. Finally, the vaginal smear can be a good guide for continuing hormonal therapy and for accurately predicting whether such therapy will be successful. In this practical conclusion for the obstetricians Pundel sets forth the following five rules, and I quote:

1. If the vaginal smear is normal in a pregnant patient, she has over 99 per cent chance of having a living infant at term.
2. If the vaginal smear is abnormal in a clinically normal pregnant patient, hormonal treatment should be started immediately, because abortion will occur later without treatment in over 30 per cent of the cases.
3. Hormonal treatment for threatened abortion is not necessary if the vaginal smear is normal. In these cases a careful clinical examination has to be done in order to discover the possible organic reason for any bleeding or pains.
4. Hormonal treatment should be administered only in such pregnant patients in whom the vaginal cytology is abnormal. The chosen hormone treatment only has chances for success if the vaginal smear returns to normal. This chance is better the less marked the cytological abnormalities are and the earlier the treatment is started.
5. Absence of a good cytological response, especially the occurrence of a typical estrogenic epithelial reaction under estrogen treatment, is a valuable sign for the death of the fetus. The prognostic accuracy of the vaginal smear is higher than that of biological or chemical hormonal assays, since substitutive hormone treatment can produce pseudonormal hormonal excretion in spite of the death of the fetus, or the biological pregnancy tests can remain positive for a while. It can be concluded that vaginal cytology is actually the best control test, not only for the course of pregnancy, but also for the effect of hormonal therapy of threatened abortions.

As indicated before, the most important indication of threatening abortion is an increase of the eosinophilic index and karyopyknotic index with concomitant decreases in the navicular cells and a decrease in the inclination to form clumps. This of course is only valuable in those smears where a normal pregnancy smear has existed from the onset of pregnancy and thus has no value in those comparatively few cases where the so-called estrogenic type of pregnancy smear is persistent throughout the pregnancy. Dellepiane[9] feels than an eosinophilic index higher than 35 per cent with navicular cells, some red blood cells, abundant mucus and predominant superficial cells are the characteristics of threatened abortion. When in addition to the above

findings basal cells in great number are accompanied by cervical and endothelial cells and by histiocytes, he considered the abortion well under way and any medical treatment as futile. The principal difference between the inevitable or already progressing abortion and the threatened abortion for which some hope of medical therapy still perhaps exists is the appearance of the small, glycogen-rich basal cell resembling the postpartum cell, which in this case has been called "abortion cell." Ferreira[10] speaks of two cytological patterns characteristic for abortion. The first is characterized by an increase of over 10 per cent in the eosinophilic and karyopyknotic indices. The second, which often follows the first picture, is characterized by replacement of the superficial and intermediate cells by parabasal and postpartum cells. In artificially induced abortion the second pattern appears later. Papanicolaou[24] depicts in his Atlas two cells which he is tempted to call abortion cells. They preserve the navicular shape but are acidophilic and have a small pyknotic nucleus which shows signs of fading. Smolka and Soost[41] warn that the cytological diagnosis of abortion is fraught with considerable difficulties since cell pictures change very rapidly and since the blood usually present is a greatly disturbing factor. They also point to the appearance of trophoblasts and syncytial cells in the case of incomplete abortion, which of course are sufficiently different from the usual vaginal cytology to be recognized easily. Unfortunately their occurrence is extremely rare. Stamm and co-workers[42] also agree that an elevation of the eosinophilic and karyopyknotic indices is the most frequent expression of hormonal disturbance in threatened abortion or threatened premature labor and recommend the estrogen test as a means to determine the exact nature of the hormonal disturbance. They compared the eosinophilic and karyopyknotic indices in such cases with the urinary excretion of pregnanediol and estriol and found that it was impossible to interpret an increase of the eosinophilic index alone as an indication of either relative or absolute luteal insufficiency. They also found that cases with low estriol excretion usually had high eosinophilic and karyopyknotic indices and finally showed that the majority of cases with high eosinophilic and karyopyknotic indices had an estrogenic insufficiency without luteal insufficiency, whereas cases of threatened abortion with normal cytology usually have a normal estriol excretion. A sufficient estrogen stimulation in the presence of sufficient progesterone seemed to them therefore an essential requirement for a smear showing normal pregnancy cytology. These startling conclusions, based upon the examination of 19 cases of threatened abortion and one experimental administration of hormones to a menopausal woman with cancer of the breast, certainly need further confirmation.

In order to avoid the difficulty caused by the admixture of blood in the vaginal smear many workers have tried to use the urocytogram as a means of diagnosing threatened or established abortions. Langreder and Merker[18] were unable to find postpartum or abortion cells in their urinary cytology studies of incomplete abortion, whereas Ramos suggests an abundance of superficial cells (more than 40 per cent) and a scarcity of navicular cells as signs of threatened abortion due to progestational deficiency. He considers the appearance of from 20 to 60 per cent basal cells in the urinary sediment a sign of combined estrogen and progesterone deficiency. In cases of fetal death he describes a "dirty smear" with more than 50 per cent basal cells, considerable cytolysis of the intermediate cells, and abundant leukocytes and histiocytes.

Using a Millipore technic we[21] examined 100 catheterized urine specimens from 100 patients with a diagnosis of incomplete

abortion verified by histological examination. These were compared with the urines of 100 nonpregnant women of all ages undergoing various types of surgical procedures. Twenty-five per cent of the urines of aborting women showed characteristic postpartum cells, whereas only 5 per cent of the controls showed similar cells. Only in two specimens could we find the abortion cells described by Papanicolaou; none were found in the controls. There were no other outstanding findings which permitted us to differentiate the urocytograms of the two groups. For this reason we feel that the presence of postpartum cells is perhaps the best proof of fetal death that we have at the present time, although certainly they are not present in the majority of the urines and occasionally appear in the urine of a nonpregnant individual.

With regard to the prevention of a threatened abortion, opinions are quite unanimous that the vaginal smear can be of extreme value in choosing the right therapy and in predicting its success or failure. As stated before, the administration of estrogenic material to women with normal pregnancy smears will not alter the vaginal smear. In patients with abnormal pregnancy smears suggesting a hormonal imbalance, the administration of hormones will be quickly reflected in the vaginal smear. Our experience during the past years has shown that progesterone is not the only hormone which when administered can prevent a threatened abortion. Early in 1940 Smith, Smith and Pundel[38] reported better results by the injection of progesterone and estrogen, and it is obvious that the animal experiments upon which the abortive effect of estrogens was based have no value for the human pregnancy. Vaginal cytology is an extremely sensitive test for study of the effect of hormonal treatment in threatened abortions. It permits us to follow daily the equilibrium between progesterone and estrogens. It is important first to establish the severity of the hormonal deficiency. This is best done by doing daily vaginal smears for four days. The first appearance of red blood cells should be a warning signal that abortion may be imminent. We know that progesterone has a soothing action on the myometrium, which is an important factor in the development of young pregnancies. We also know that it has a direct action on the nidation of the egg and its embedding. Estrogens produce proliferation of the uterine elements and possibly stimulate the intrinsic production of estrogens by promoting and maintaining the corpus luteum. Estrogens also may stimulate progesterone production by the placenta, according to Smith, Smith and Schiller.[39] For this reason obstetricians like Pundel[34] propose administration of estrogens and progesterones together in cases of threatened abortion. If the eosinophilic index of the vaginal smear decreases under the effect of this treatment and returns to normal, the pregnancy has a good chance to survive. If the eosinophilic index is not influenced or continues to increase, then the chance for preservation of the pregnancy is very poor and expulsion of the fetus seems inevitable. In this case the vagina has regained its normal reactivity against estrogens. If on the other hand the eosinophilic index decreases and a large number of basal cells appear, then fetal death can be diagnosed and abortion seems inevitable. It is indeed unfortunate that to the present the vaginal smear is not considered to reflect the effect of gonadotropic hormones in spite of the fact that it is the most sensible, easiest and most economical test for the study of threatened abortion and its therapy.

Other pathological forms of pregnancy which may be reflected in the vaginal smear include ectopic pregnancy, hydatidiform mole, and toxemias of pregnancy. Ectopic pregnancy, in addition to its usual clinical significance, will produce a pregnancy

smear once the condition has existed for more than two months. However, this smear very quickly will show a rise in the eosinophilic index whenever the ectopic pregnancy reaches its natural termination by rupture. Arrighi[2] suspects this condition when a strongly progestational smear with a variable number of red blood cells is present. Bickenbach and Soost[3] recommend the vaginal smear as a differential diagnostic sign between ectopic pregnancy and an inflammatory ovarian mass or cyst. De Brux[8] thinks it is very important to follow up with daily smears whenever ectopic pregnancy is suspected and denies that it is possible to designate a precise type as the smear of ectopic pregnancy. With regard to hydatidiform mole, only a few reports are available. Pundel[35] describes the appearance of Langhans type cells together with multinucleated syncytial elements. In addition, the pregnancy smear was of the pure estrogenic type without any evidence of progesterone activity.

Hyperemesis gravidarum and other forms of toxemia of pregnancy cast no reflection in the vaginal smear or the urocytogram, according to the few reports available.[36, 37] We agree with Pundel, who argues that if a hormonal basis should be responsible for this condition it is entirely different from that dealing with spontaneous abortion and that it probably involves the production and utilization of gonadotropins which cannot be demonstrated with the vaginal smear.

Summary and Conclusions

1. The recent achievements of exfoliative cytology in the study of normal and abnormal pregnancy have been reviewed.

2. The diagnosis of pregnancy by the vaginal smear method is feasible but impractical since quicker and more reliable methods are available.

3. The typical vaginal smear of pregnancy can be found in 60-77 per cent of pregnant women after the third month. It is characterized by clusters of basophilic navicular cells, a low eosinophilic index (below 5 per cent) and a low karyopyknotic index (below 10 per cent).

4. Cytolytic smears are due to overgrowth of the Döderlein bacillus and make a cytological evaluation impossible, but this does not represent a prognostically unfavorable sign. Such smears are easily converted into the noncytolytic type by the administration of antibiotics.

5. The investigations of Lichtfus, Pundel, and others stress the importance of cytological examination near the term of pregnancy, and according to these workers the onset of spontaneous labor is predictable within a period of ten days. The appearance of basal and parabasal cells in large numbers signifies placental fatigue and demands the immediate initiation of labor.

6. After termination of the pregnancy the vaginal smear is characterized by the appearance of glycogen-rich basal and parabasal cells in large numbers which persist during the lactation period.

7. Abnormal vaginal smears during pregnancy possess a high diagnostic value for the recognition of hormonal abnormalities, threatening abortion, and fetal death. Additional work is urgently needed to establish this fact on a larger material than is available now.

8. The urocytogram during pregnancy reflects similar changes as the vaginal smear. It has the advantage of being less influenced by infection and hemorrhage but has the disadvantage of less sensitivity.

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Ninth Annual Meeting 1961—The Inter-Society Cytology Council

The ISCC will meet at the Hotel Peabody, Memphis, Tennessee on November 2, 3 and 4, 1961. The Scientific Program will include a panel on November 2, moderated by Dr. Howard W. Jones, Jr.; several eminent authorities in the field will discuss cytogenetics in medicine, with technics of interphase and metaphase analysis; the clinical significance of autosomal and sex chromosomal abnormalities and aberrations of the sex chromatin body, and relationships to cancer. The following day Dr. George O. Gey will chair a symposium on cell modulation, maturation and neoplastic transformation, with a consideration of the role of extrinsic and intrinsic factors which induce cellular changes in normal and disease states. Several authorities in the field will take part in this symposium. The third day will be highlighted by an afternoon seminar under the direction of Dr. Leo Koss. Interesting cases and problems in cytology will be presented and diagnosed by Dr. Koss and his panel. Printed histories and photographs of the cellular preparations will be distributed prior to the seminar.

A round-table luncheon will be held on November 3.

At the Ninth Annual Business Meeting on November 2, the Medical Members will vote upon the proposed amendments to the Constitution and By-Laws which were read at the Eighth Annual Meeting in Chicago last September and were sent in writing to all Medical Members in December as an enclosure with the last issue of the *Cytology Newsletter*. The amendments in summary will change the name of the Council to American Society of Cytology; will decrease the number of officers; and will eliminate the three categories of Medical Membership, that is, Clinician, Cytologist and Pathologist.

In Memoriam

Professor Arnaldo De Moraes
1893-1961

Arnaldo De Moraes was born on August 28, 1893. After finishing college in 1910, he went on to complete medical studies at the Medical Faculty of Rio de Janeiro in 1915, having been one of the top students in his class throughout his studies. As a student, and later as an assistant, Dr. De Moraes worked for the University Department of Obstetrics. He also assisted at the Hospital Misericordia, and the Hospital Pro-Matre. During this period Dr. De Moraes was an associate of the famous surgeon Professor Brandão Filho. In 1920, Dr. De Moraes was awarded the position of Inspector of Health of the National Public Health Department. A Rockefeller Foundation grant enabled Dr. De Moraes to study at several hospitals and universities in the United States in 1927; he visited Johns Hopkins University, Harvard University, Cullen's Gynecologic Clinic, and William's Obstetrical Clinic. Later he continued his studies in Austria, France and Germany.

Dr. De Moraes was an officer of the Legion of Honor, and was a member of many medical societies, among them, the National Medical Academy of Brazil, the Medical Academy of Buenos Aires, the American College of Surgeons, the Gynecological and Obstetrical Societies of Montevideo, Chile, Paraguay, and Paris. He was a founding member of the Brazilian College of Surgeons, the Brazilian Gynecological Society, the Brazilian Society of Sterility, and the Brazilian Society of Cytology, of which he was Honorary President.

From 1936 on, Dr. De Moraes represented Brazil at many International Medical Congresses in South America, North America, and Europe, and was Honorary President, French Congress of Gynecology in 1949.

After having been Titular Professor of Gynecology of the Faculty of Niteroi for some years, in 1935 Dr. De Moraes was called upon to be Chairman of the University Department of Gynecology at Rio de Janeiro. There he assembled a team of well-trained specialists, among them six Associate Professors, who for 25 years assisted him in teaching post-graduate and regular courses. In 1942, Professor Arnaldo De Moraes established in his hospital the first department in Brazil for the treatment of sterility in women, and in 1948, he again led the way in Brazil by using cytology and colposcopy for the detection of female genital cancer.

Among Professor De Moraes' outstanding achievements are the establishment of the Institute of Gynecology in 1947, the foundation of a Private Maternity Hospital in 1930, and the creation in 1936 of an international gynecological journal, "Anais Brasileiros De Ginecologia," which became the official organ of several societies.

In October of 1960, Professor De Moraes, now Dean of the Medical Faculty of the University of Rio de Janeiro, celebrated 25 years of service as a Professor. Until his death, April 6, 1961, Professor De Moraes devoted his health and energies to his two great loves, teaching and the fight against cancer.—Clarice Do Amaral Ferreira.

Symposium on Probable or Possible Malignant Cervical Lesions — Carcinoma *in Situ*

I. Histology of Carcinoma *in Situ* (Continued from July-August Issue)

What Is *Not* Carcinoma *In Situ*?

Friedrich Bajardi

Graz, Austria

In the author's investigations an attempt has been made to eliminate such suspicious lesions of carcinoma *in situ,* which might possibly show a regression without any treatment. On the other hand, it must be taken into consideration that such changes can remain stationary for years and even decades, or that carcinomas may yet develop in places showing such suspicious lesions. A development in this direction is, however, not inevitable.

In order to distinguish lesions of this kind morphologically, a great amount of experience is required in many cases. It must, however, also be admitted that a definite evaluation of changes in the epithelium based on histological criteria is not possible in every case, and the personal attitude of the investigator will often be decisive in reaching the final diagnosis.

Among the suspicious pictures we first distinguish those in which the suspicion is at least partly due to technical defects of the section. In this respect horizontal or oblique sections should be mentioned. If the histological section is cut, for instance, through the basement membrane of a normal or almost normal squamous epithelium without also reaching the upper layers, the picture of an epithelium with relatively numerous cells will result. The nuclei are hyperchromatic and occasionally somewhat polymorphic in their appearance; mitoses can also be observed. On the other hand, the cell borders are usually still distinguishable. In Figure 1 some epithelial buds, belonging to an "abnormal" squamous epithelium, are cut horizontally. The picture already resembles in some way that of cervical glands filled with carcinomatous epithelium. The evaluation becomes more difficult when horizontal sections are placed through the base of an epithelium showing an atypical basal hyperplasia or a dysplasia. It becomes still more difficult when cervical glands which are filled with the above kind of epithelium are cut horizontally. Here we find pictures which can no longer be distinguished from a classical carcinoma *in situ.* A reliable evaluation of these changes is only possible by means of serial sections, which make the upper layers of the epithelium visible also.

However, both basal hyperplasia and dysplasia may cause difficulties with regard to a diagnosis, even in cases where the sections are technically perfect. In the presence of an atypical basal hyperplasia, we consider the change as carcinoma *in situ* only when the hyperplastic part, showing cytomorphologically the symptoms of an undifferentiated carcinomatous epithelium, has seized the whole extent of the epithelium, with the exception of some two or three of the uppermost layers. The lesions in Figures 2 and 3 were considered as not yet being malignant dysplasias. It is true that in the epithelium in Figure 2 there appear an especially large number of cells, while the nuclei show faint granulations and, as a rule, distinct nucleoli. On the other hand, the nuclei are only slightly polymorphic, the cell borders being nearly always distinguishable. Moreover, a maturing of the cells can be observed, gradually progressing from the base to the sur-

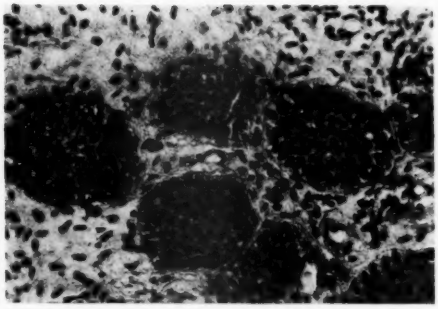

FIG. 1. Horizontal section of buds in an abnormal epithelium.

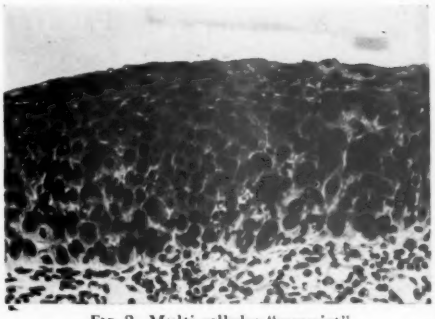

FIG. 2. Multi-cellular "unquiet" squamous epithelium.

face. A giant cell having numerous nuclei, shown in the right section of the picture indicates that the generally increased potency of cell division has been disturbed (separation of nuclei without division of cells). The pronounced polymorphic epithelium, as shown in Figure 3, is also partly attributed to the great number of giant cells bearing numerous nuclei. Especially striking, however, are some large hyperchromatic nuclei without any structure, situated just below the surface of the epithelium. The cell borders are again nearly everywhere clearly visible. In a case of such a "degenerative polymorphism," a complete regression could be proved, not only cytologically and colposcopically, but also histologically. This change had originally been described by other investigators as carcinoma *in situ*. Both in Figures 2 and 3 we gave the diagnosis as "unquiet epithelium." We have shown a certain reserve in giving a diagnosis of carcinoma *in situ*, even in cases of a still more pronounced pathological epithelium. This is proved by a microphotograph, Figure 4 on page 346, which depicts the "atypical epithelium."

Special mention must also be made here with regard to the "undifferentiated regenerating epithelium" (Figure 4). This epithelium is composed of young, highly proliferating cells. Their nuclei are mostly hyperchromatic and occasionally already show a high degree of polymorphism. This was observed particularly when such an epithelium covered a minor ulcer or an erosion. Mitoses may be found in increased numbers, and the cell borders occasionally blurred. Such an epithelium, however, is nearly always distinctly thinner than the epithelium of a carcinoma *in situ*.

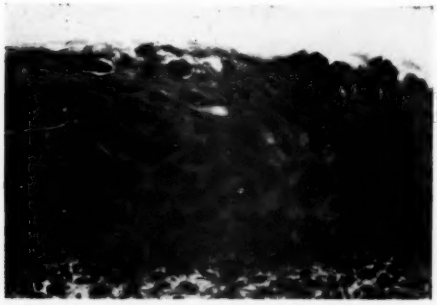

FIG. 3. "Unquiet" squamous epithelium (unruhiges Epithel) with pronounced cellular polymorphism.

FIG. 4. Undifferentiated regenerating epithelium showing pronounced cellular polymorphism.

Finally, we must distinguish those cases already showing an early invasion of carcinomatous epithelium as against a carcinoma *in situ*. It can well be understood how difficult the evaluation of these changes may be. A full report on the morphology of an early invasion is, however, not possible within the scope of this paper, because of the great variety of the respective histological details.

MARCEL G. GAUDEFROY

Lille, Nord, France

IN a round table discussion, it is very difficult to come to an agreement on a case of so-called carcinoma *in situ*. One thinks: it is not a carcinoma *in situ;* another: it is an early invasive carcinoma or a micro-cancer. Observations of *in situ* carcinomas cured by aureomycin have been published. Evidently, one is not speaking of the same thing!

Perhaps it will be easier to define carcinoma *in situ*, having eliminated some aspects which are surely not "*in situ* carcinoma."

In order not to abuse the hospitality of *Acta Cytologica*, I present five photomicrographs accompanied by comprehen-

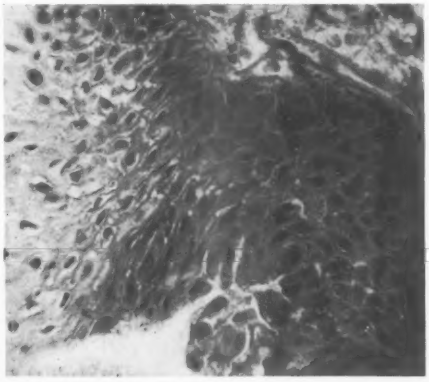

FIG. 1. Sudden change of an ectocervical epithelium with, on one side (right and below), evident dysplasia, insufficient cellular maturation, and on the other side (left and above), clearer and higher epithelium, irregular architecture, nuclear abnormalities, showing suspect changes, but with perinuclear vacuolization and strongly decreased charge of glycogen. Smear: Class III/IV of Papanicolaou.

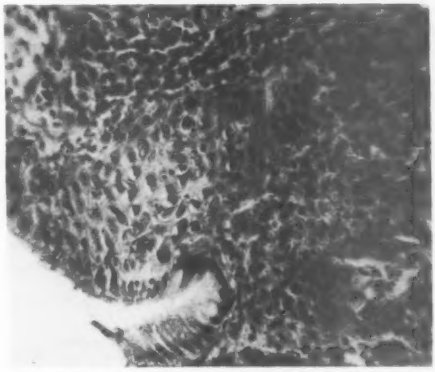

FIG. 2. At the ecto-endocervical junction, near the normal glandular-endocervical epithelium, at one point, a squamous epithelial zone sinking into the subjacent chorion, with a very irregular architecture, dyskaryotic nuclei of varied size and shape, no clear limit from the connective tissue, which is markedly infectious. Smear: Class III of Papanicolaou.

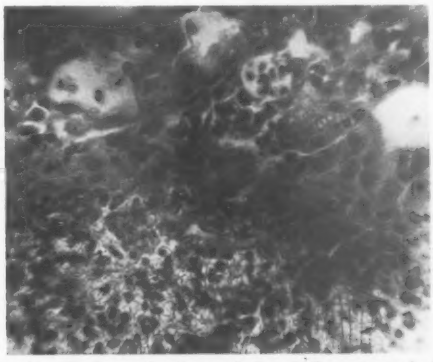

FIG. 3. Irregular hyperplasia of the ectocervical epithelium, disappearance of the basement membrane, mixed immature and mature elements, large vacuoles or pseudocavities with polymorphonuclears, some active nuclei and others, degenerative or pyknotic. Smear: Class IV of Papanicolaou.

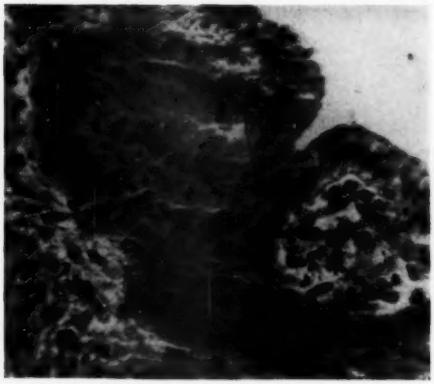

FIG. 4. Under the normal columnar epithelium, squamous zone creeping from the ectocervical epithelium, with marked dysplasia, disappearance of the normal squamous architecture, dyskaryotic and irregular nuclei. It is possible to recognize the columnar epithelium showing a curved line under the glandular aperture (left and above), and the malpighian atypical cells surrounding that curved columnar zone. Smear: Class III/IV of Papanicolaou.

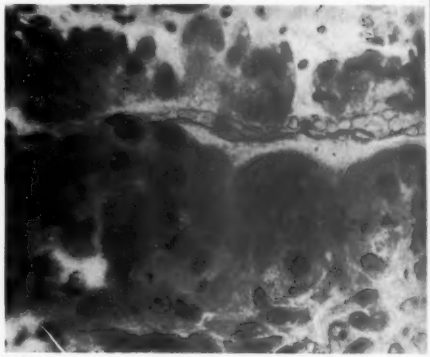

FIG. 5. Upper portion: endocervical columnar epithelium. Lower portion: very atypical ectocervical epithelium, with completely depolarized cells, irregularly mature nuclei, increased chromatin, no clear limiting basement membrane, and intercellular polymorphonuclears. Smears: Class IV of Papanicolaou.

sive, but carefully restricted comments (all are cervical biopsies).

In all five cases, there were present on old vaginal trichomoniasis, plenty of intercellular or intracellular polymorphonuclears, and strong infiltraion of polynuclears, lymphocytic and plasmocytic elements into the surrounding connective tissue. With the aid of a special staining technic (alcian blue—P.A.S.) taught to me by Lindenschmidt of Heidelberg, Germany, I think, as the Heidelberg Gynecological School, those atypical aspects are inflammatory reactive processes to the Trichomonas infestation. Surely they are not carcinomas *in situ*. All these cases are cured for five years by anti-Trichomonas treatment and a simple electrocoagulation of the cervix.

RAIMUND KRIMMENAU

Dresden, Germany

THERE is generally agreement about the diagnosis of an invasive carcinoma. However, the diagnosis of a carcinoma *in situ* is not generally agreed upon.[8] Epithelial changes deviating from physiological variants of the ectocervical and endocervical epithelium are found extremely often. These are *by-products* of the efforts for a pre-diagnosis of carcinoma, i.e., the "by-products" obtained by Schiller, Hinselmann and cytology.

Starting from the "classical" definition of Schauenstein (increase stratification, cellular borders hardly, or not at all, discernible, hyperchromasia and polymorphism of the nuclei, increased mitosis), there are pathological changes (with their numerous variants) which can be confused with carcinoma *in situ*, thus:

A. All secondary squamous epithelial formations based on a so-called "epidermization" in

FIG. 1. Fresher epidermization with remnants and large areas of columnar epithelium. This was identified in another place as "squamous cell carcinoma."

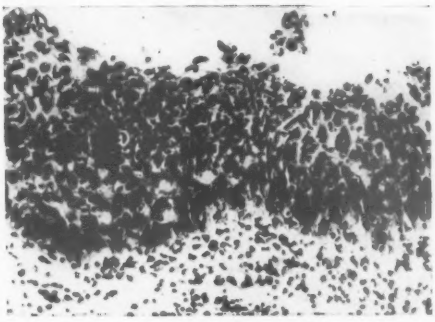

FIG. 2. Increased proliferating epithelium penetrated by leukocytes with increased stratification, hyperchromatic and somewhat polymorphic nuclei. The diagnosis "inflammatorily irritated epithelium" was given by us only after inspection of the serial sections. (Cytology positive.)

the original sphere of columnar epithelium.

B. Inflammatory epithelial alterations.

C. Combination of A and B (particularly frequent).

D. So-called "dysplasias."

A. This is the false positive diagnosis which is frequent among less experienced examiners (Fig. 1). Old processes of this kind correspond to a "simple atypical epithelium" (Hinselmann, Class Ic or Ib). Fresher metaplasias permit the clear recognition of the large areas or remnants of columnar epithelium, beside undifferentiated cell proliferations.

B. With inflammations, especially chronic ones, one finds sometimes an increase of stratification with greater epithelial proliferation and polymorphism and hyperchromasia of the nuclei. In our opinion a safe differential diagnosis of such changes is possible only if the complete alteration and its normal environment is available (Fig. 2).

D. Dysplasia has only a quantitative difference from carcinoma *in situ* by superficial maturation, preserved stratification and distinct cell borders. Superficial parakeratosis is frequent. According to our experience, in the neighborhood of the lesions, invasive growth was frequently found (the growth potential becomes evident only with the invasion). We refuse to assume an inflammatory genesis of such alterations without histologically existing findings of inflammation, such as found by Bechthold and Reicher.[2]

We always demand, except for cases B, C, and D, that we see the totally changed area in a normal environment (mostly by conization) in order to be diagnostically certain that carcinoma *in situ* has been ruled out.

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CLAUD W. TAYLOR

Birmingham, England, U.K.

THE EPITHELIUM of the cervix shows variations due to pathological conditions and physiological changes; these variations sometimes are mistaken for carcinoma *in situ*. When biopsy has been undertaken following a positive or suspicious cytological report, there is a tendency to regard any unusual epithelial change as some form of carcinoma; in general this will be true but not always. This is no reflection on the value of cytology but rather to emphasize that the ultimate value of cytology depends on accurate histology. Bias in interpretation yields no service to either field of pathology.

Epidermization, replacement of columnar epithelium by squamous epithelium, is unlikely to be mistaken for carcinoma *in situ* when the process is regular and orderly. When the process is irregular and shows a mixture of mucus-secreting and squamous cells the pleomorphic pattern may be mistaken for carcinoma *in situ*. Low power inspection shows bizarre epithelium both on the surface and in glands but critical examination shows maturation of the epithelial cells, both squamous and mucous. The pleomorphic nature of the cells is due to cytoplasmic rather than nuclear changes.

Proliferation of the basal cells of the stratified epithelium of the cervix is common with erosion, ectropion, inflammation and pregnancy. The basal cells permeate the higher strata of the squamous epithelium and due to their greater comparative nuclear content cause the affected portion of epithelium to stain darkly with hematoxylin. This abnormal epithelium may be demarcated from the normal epithelium by a so-called Schiller line. At first sight, therefore, affected strips of epithelium resemble carcinoma *in situ*. Closer inspection, however, reveals dissimilarities. In basal cell hyperplasia there is always a tendency for the cells to mature and produce stratification. This maturation may be obvious, when no confusion should arise, or may need careful study for recognition. It is unusual for the basal cells to occupy fully more than the lower half of the thickness of epithelium; above this level many of the cells show cytoplasmic maturation with diminution in nuclear activity. Nuclear degeneration can be mistaken for mitosis; although mitoses may be numerous in the lower half of the epithelium they are absent or few in number in the upper zones. The difference between basal cell hyperplasia and carcinoma *in situ* lies in the effort of the former to produce a tissue with some degree of purpose whereas the latter appears as uncontrolled cellular growth.

For descriptive purposes the distinction between basal cell hyperplasia and carcinoma *in situ* is clear. In practice it is at times extremely difficult and one lesion may merge into the other. When the histological report decides the fate of the patient the pathologist may be inclined to diagnose carcinoma *in situ* because he is unable to be sure that the lesion is not carcinoma *in situ*. Pressure in coming to a decision often influences the report which once made becomes fact and may be included in statistics. It is not possible always to be sure of the histological diagnosis. The label "equivocal carcinoma *in situ*" may be helpful in these instances. The patient can be treated but classification of the lesion must be in abeyance.

Epithelial proliferations in the cervix during pregnancy cause confusion, but have been overemphasized in relation to carcinoma *in situ*. The histological criteria of diagnosis are the same whether or not the patient is pregnant.

Occasionally an early superficial invasive carcinoma is misinterpreted histologically as carcinoma *in situ*. The epithelial changes are often quite different from characteristic carcinoma *in situ* and may show cell nests; undoubted stromal invasion is present. The whole lesion may constitute stromal infiltration but because of the superficial site it may be mistaken for carcinoma *in situ*. Not every carcinoma can be traced to arise from a pre-existing *in situ* lesion.

Discussion

Anthony F. Anderson, Edinburgh, Scotland, U.K.: To Bajardi: Let me confess that I would call his Figures 2 and 3 malignant—so far as one is justified at all from photographs. But I do not follow him when he states a complete regression "could be proved" etc. If biopsy is repeated and nothing found, does he regard this as proof of regression?

May I split hairs with Gaudefroy? He says "evidently one is not speaking of the same thing." Surely the explanation is, as has been said so often, that the labelling of a lesion from microscopic examination is exceedingly subjective, and that we *are* speaking of the same thing. It is we who differ, not the tissue lesion, and we will continue to differ. To talk of cases "cured" by simple (why simple?) electrocoagulation seems to me naive; the lesion, whatever it is, may be destroyed by cauterization and many such must have been satisfactorily dealt with in this way without ever having been diagnosed at all. With a Class IV smear and one of these pictures would Gaudefroy treat as he outlines and discharge the patient for good, or follow carefully? Has he the courage of his convictions?

Krimmenau's pictures defeat me in this proof medium. I note that Figure 2 was associated with positive cytology, but there is no hint that the fresher and unshrunk cell picture of cytology may help in any way.

I am delighted to welcome Taylor to this *in situ* controversy, and he has evidently gone through the usual gestation period until he is impressed with its potentialities. In all fairness, however, he might acknowledge that bias is no prerogative of those who have been at it for many years from the cytology aspect. In pathologists "biased" against cancer *in situ* (and cytology) there is a tendency to regard any unusual epithelial change as not malignant even if there is positive cytology. Who is completely unbiased, when experienced pathologists differ about invasive neoplasms, let alone non-invasive? I do not follow his idea that differentiation or "purpose" helps us with basal cell hyperplasia. Does differentiation rule out malignancy? I prefer to use the term hyperplasia (of this degree) when the nuclei are normal, and anaplasia when they appear to be malignant but the top layers are normal. After all, the full thickness *in situ* changes must have had a stage when they were only half thickness, only we don't yet push our logic that far into our management. McKelvey said all this years ago. We agree entirely with his label "equivocal" cancer *in situ*, and often use the word "borderline" ourselves. We agree about the criteria whether pregnancy exists or not, but as Novak said there is no hurry and if there is "pressure" anywhere it should be from cytologist and pathologist to the clinician to wait, rather than vice versa.

His last paragraph about early invasion is excellently propounded by Fidler and Boyes. They are ahead in Vancouver on this.

Hans F. Bettinger, Melbourne, Australia: I agree with Bajardi that none of his photographs shows a carcinoma *in situ*. I entirely agree with Taylor's exposition, except that I prefer the term "dysplasia" to basal cell hyperplasia."

Jorge Campos R. de C., Lima, Peru: To differentiate between what is and what is not a carcinoma *in situ*, experience is needed; also the number and kind of slides one examines is important because it is not rare to see cases where one slide shows a "typical" carcinoma *in situ* but new slides from the same case show areas of infiltration.

Unfortunately we must agree, at the moment, with Taylor when he states "it is not possible always to be sure of the histological diagnosis." We need to develop new histological criteria established on solid bases to differentiate carcinoma *in situ* from different kinds of cervical dysplasias. Another way to learn would be to follow up these patients without treatment. Unfortunately clinicians do not always like to do this.

Hans Limburg, Homburg, Saar, Germany: There is general agreement with the statements of Bajardi. However, in Figures 1 and 2 it should be remembered that a carcinoma *in situ* arises from the altered basal layer and thus may push the old epithelium in front of it, so that by no means must the entire thickness of the epithelium be altered. The so-called borderline or observation cases come from these types of alterations, where two-thirds or three-fourths of the epithelium is replaced by atypical cells. Multinucleated cells may evolve in inflammations by regression. It should be borne in mind, however, that such cells in Bowen's disease, under the picture of "clumping cells," may have a definite significance. In particular the borderline cases require a thorough investigation by histological serial sections.

To Gaudefroy: I entirely agree with the statements. The cases referred to in this paper mislead some investigators in the United States, and Europe as well, to the false conclusion that Trichomonas vaginitis or cervicitis causes, or at least favors, the evolution of cervical carcinoma. Considering the frequency of Trichomonas infestations, a carcinoma *in situ* may well be found at the same time. I also agree with Taylor's paper.

To Krimmenau: There is no agreement at all among the investigators as to the appearance of an early invasive cervical carcinoma.

Marco Marcov, Stara-Zagora, Bulgaria: I agree with Bajardi and Gaudefroy that not every suspicious lesion of the cervix must be understood as carcinoma *in situ*.

The epithelial cells of the cervix show some variable changes, especially during inflammatory processes, which are very similar to the picture of carcinoma *in situ*, but they return spontaneously to the cytological characteristics of normal cells.

When the inflammatory process is prolonged, we are confronted with the serious responsibility of continuing to observe the patient carefully.

In our experience we have had five patients with chronic inflammatory processes which were very suspicious for carcinoma *in situ*. Four of them returned to normal, however, at the end of the fifth year, one gave signs of carcinoma *in situ*.

In conclusion, every atypical epithelium, not known to be a carcinoma *in situ*, must remain under careful observation.

Rodolfo Sammartino, Buenos Aires, Argentina: Carcinoma *in situ* is generally considered an "irreversible" lesion and also potentially malignant. Therefore, it is almost as important to separate all that which is *not* carcinoma *in situ* as it is to recognize what is carcinoma *in situ*. We are conscious of the impossibility of achieving this in practice due to lack of infallible objective criteria.

I am in disagreement with the opinion that adjudges as carcinoma *in situ* only such alterations as invade the entire thickness of the epithelium. The "carcinoma *in situ*" that does not reach the upper surface is called variously—"atypical basal hyperplasia" by Bajardi, and "dysplasia" by Krimmenau, but the quantitative difference does not change the potentially malignant character of the epithelium and hence, such a dual criterion is not understandable. I would insist: carcinoma *in situ* is due to an anarchical lineage of cells, without any consideration of number.

Thus, all epithelial growths, with morphological or functional changes that show rather the character of abnormality, i.e.: aglycogenic epithelium diffuse hypertrophy, basal hyperplasia, inflammatory regeneration, keratosis and parakeratosis, epidermization, metaplasia, are *not* carcinoma *in situ*.

Now I wish to put the following question to the main speakers: What is the carcinomatous membrane adjacent to an invasive carcinoma—is it a kind of superficial propagation, or is it an independent carcinoma *in situ*?

Wolfgang Walz, Heidenheim, Brenz, Germany: The presentation of Krimmenau probably best embraces all the changes which are not yet carcinoma *in situ* but which in a quantitative regard may display all the atypias which make a differential diagnosis other than carcinoma *in situ* more difficult.

Concerning Group A by Krimmenau, I have nothing to add.

To Group B: In an inflamed epithelium there always occurs an invasion of lymphocytes and leukocytes. Consequently, the basal cell layer may be markedly disturbed by the inflammatory influences. In most cases the intercellular spaces are widened, the cells themselves are often vacuolized and the nuclei markedly changed. However, the latter are not as rich in chromatin as those in a carcinoma *in situ* or in a dysplasia.

To Group D: Here special attention has to be paid to the basal layer. As long as this is arranged in a palisade-like formation and the cells resemble normal basal cells, in my opinion we still deal with a dysplasia. This is very well illustrated in Figure 3 in the paper by Bajardi. Differentiating a carcinoma *in situ* solely by the higher portions of the epithelium is not justified, in my opinion, since in both dysplasia and carcinoma *in situ* there may be a tendency towards maturation which may lead to cornification.

Closing Remarks

Claud W. Taylor: There seems to be less difficulty in recognizing what is not carcinoma *in situ* than what is carcinoma *in situ*. Anderson raises, among other things, the question of differentiation of "purpose"; to a pathologist purposeless growth is one of the characteristics of neoplasia in contrast to the purposive growth seen in repair, response to irritation or hypertrophy. Differentiation of cells certainly takes place in neoplasia but the growth remains "purposeless." We have to attempt to distinguish "dysplasia" from "neoplasia" on general principles and reiterate the point noted by Walz that "there are no specific features known for the single carcinomatous cell." We agree with Sullivan that further elaboration on the formation of cell nests in carcinoma *in situ* would be of value.

Friedrich Bajardi: To Anderson: Semi-serial sections on more than 200 ring and cone biopsies have shown that not only the carcinoma *in situ*, but also the unquiet ("unruhige") and the atypical epithelia are multicentrically developed in the vast majority of the cases. Thus, the possibility of removing such a pathological epithelium by a simple ring biopsy is limited. This holds especially true for the small (punch) biopsies. As a matter of fact, in that case of regression the pathological epithe-

lium was discovered in a punch biopsy. Therefore, it seems much more likely to us that regression occurred rather than that the complete lesion was removed by the small biopsy. The criticism on my Figures 2 and 3 did not come unexpectedly to me. On the contrary I was surprised by the predominancy of the agreeing opinions by some discussants. Certainly we deal here with changes in which the subjective views of each examiner will influence the diagnosis to a certain degree.

To Limburg and Sammartino: We agree with their opinion that the atypical basal hyperplasia may have already the same potential as the carcinoma *in situ*. On the other hand the possibility cannot be ruled out that the hyperplastic cells will still differentiate, however retarded, and then only in the upper third of the epithelium. This is the reason for our own restraint in the evaluation of the atypical basal hyperplasia, although the diagnosis "carcinoma *in situ*" seems to be very close at hand.

To Sammartino: In regard to the "atypical non-invasive zone" around an invasive carcinoma, I might refer to my closing remarks in the topic, "The Atypical Non-Invasive Zones Around Invasive Cervical Carcinoma," in this Symposium.

Marcel Gaudefroy: I am sorry the black and white illustrations were unsatisfactory, but it is impossible to show the original color slides. Moreover, I am sorry for the criticism of Anderson. Evidently we are often speaking differently of the same thing, but very certainly we are often also speaking in the same way of different things. As Limburg has perfectly understood, the cases referred to in my papers mislead some investigators to false conclusions of carcinoma *in situ*. I tried to show some lesions with difficult diagnosis. These sometimes resulted in false positive cytological reports, since in my opinion they were not carcinoma *in situ*.

Raimund Krimmenau: To Anderson: I am in full agreement that absence of recurrence after excision or cauterization of an alterated tissue does not prove absence of malignancy in the removed tissue. The purpose intended by demonstration of Figure 1 seems to have been misunderstood. The picture was not meant to demonstrate an especially questionable borderline case. It was intended to show a mistaken evaluation of even clear-cut findings within the context of the differential diagnosis in question here.

It has been emphasized by us elsewhere[1] that the cytological pattern in a given tissue change may help the histological interpretation. Unfortunately, up to today there are no specific criteria for the tumor cell.[2]

To Campos: The mere follow up observation of patients with so-called questionable epithelial changes without further therapy is for reasons dangerous on one hand stated by Campos himself ("because it is not rare to see cases where one slide shows a 'typical' carcinoma *in situ* but new slides from the same case show areas of infiltration") and the same situation may occur also in cases with "questionable epithelial changes" and does not prove anything on the other hand (who knows what's left behind?).

To Sammartino: We share, together with Limburg and Walz, the opinion that a carcinoma *in situ* may be present also in those cases where superficially normal cells are found. Highly bizarre basal anarchical patterns, however, are also found in marked inflammatory reactions so that the interpretation is rendered even more subjective as in other cases. To the question of the carcinomatous covering in invasive carcinoma: We are convinced that carcinoma *in situ* and invasive carcinoma belong to one and the same clinical entity. Otherwise all our efforts would have little practical value.

To Walz: The detailed description of inflammatory epithelial changes corresponds with our opinion.[3] However, the chromatin content may not be very marked in the carcinomatous process, whereas it may be very high in inflammatory reactions.

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Prof. Papanicolaou to Direct Miami Cancer Institute

Dr. George N. Papanicolaou, Professor Emeritus of Clinical Anatomy, Cornell University Medical College, New York City, was named director of the Cancer Institute in Miami.

After being associated with the Cornell University for over 48 years, Dr. Papanicolaou will assume his new duties as director of the Institute in October of this year. Under the directorship of Dr. Papanicolaou the main emphasis of the Institute will be devoted to cellular research. The Institute will be enlarged during the next few months by $250,000 research facilities. The address of the Cancer Institute in Miami is: 1155 NW 14th Street, Miami, Florida.

What Percentage of Cervices Show Early Invasion in Serial Histological Sections in Uteri Which Were Removed Under the Biopsy Diagnosis of "Carcinoma *In Situ*"?

WERNER BICKENBACH AND HANS-JÜRGEN SOOST
Munich, Germany

AT the Universitäts-Frauenklinik in Munich we found: invasive pattern of carcinoma 13 times on conization among 45 cases, where histological examination after punch biopsy had revealed a marked atypical epithelium, invasive pattern of carcinoma four times on conization among 16 cases where histological examination revealed a plainly atypical epithelium.

As a matter of fact only conization had been able to detect invasive growth in about 25 per cent of the cases. It is definite that simple punch biopsy is not sufficient on suspicious changes of the cervix. If the result of the first histological examination is a plain or marked atypical epithelium, a conization has to be performed and the tissue has to be examined in sections of different levels, in order to prevent overlooking a carcinoma, or intensifying therapeutic management unnecessarily. On the other hand, showing how little invasive carcinoma may be extended, we only found non-suspicious tissue on material from conization in four cases, where punch biopsies had shown unequivocally invasive carcinomas.

JORGE CAMPOS R. DE C.
Lima, Perù

ACCORDING to the statement made in a previous section of this Symposium, diagnosis of cervical carcinoma *in situ* can be made only after study of multiple radial sections of a ring cervical biopsy which should include the internal part of the ectocervix as well as the endocervix.

Frequently, after studying 28 or 30 radial cervical sections, invasive carcinoma is found in one or more zones; therefore the diagnosis of carcinoma *in situ* is discarded.

Between 1952 and 1958 we have collected 85 cases of cervical carcinoma *in situ*, diagnosed according to the above procedures. During the same period of time, another 41 cases were discarded from this group and were reported invasive carcinoma, because invasion of the connective tissue was found in some microscopical areas. This means that, according to our experience, in approximately one case of every three, the multiple radial sections of a ring cervical biopsy proved that carcinoma was already invasive.

These figures do not include the cases in which "carcinoma *in situ*" was found in a simple biopsy taken at the edge of a cervical tumor which obviously was not carcinoma *in situ*. We refer only to cases where clinical examination did not show carcinoma, and the only evidence of malignancy was obtained by a cytological routine test and/or by a cervical biopsy from a lesion which seemed to be benign.

H. K. FIDLER AND D. A. BOYES
Vancouver, British Columbia, Canada

IN studying the patterns of early invasion we have found it of value to define two stages of invasion.[1, 2] The first is the stage of discrete, micro-invasive foci in which scattered, minute aggregations of cells have broken through the basement membrane, most commonly in the depths of glands filled with carcinoma *in situ* but also from the surface lesion. In many of the foci the cells mature and may even die. During a ten year period while 504 cases of carcinoma *in situ* were investigated, 31 cases with this early type of invasion were found. Our rate of 6 per cent compares with some of those previously reported,[3, 4] but is considerably different than the 60 per cent[5] and 63 per cent[6] reported elsewhere. The wide range probably reflects a difference of opinion in what constitutes early stromal invasion.

The second stage of invasion is that of frank confluent invasion in which the lesion is relatively small and hidden to clinical observation. Histologically these cases are readily diagnosed and do not cause the difficulty in interpretation experienced in the first group. In most of these the intraepithelial portion is extensive and the invasive part relatively small and is hidden in the endocervical canal or just within the external os. During the ten-year period, 20 cases of occult invasive carcinoma were studied by serial block, step serial section technic, mainly of cone biopsies. Preliminary bite or punch biopsies had been studied in nine of these cases and showed only carcinoma *in situ* or carcinoma *in situ* with questionable invasive foci.

Because it is our practice to perform cone biopsies on all cases with positive cytology in which no suspicious target lesion is present, most of our cases do not have preliminary bite or punch biopsies. Our total material does show that in those cases thought clinically to be no more than carcinoma *in situ*, and therefore warranting a cone biopsy for complete histologic evaluation, 6 per cent showed the earliest discrete micro invasive foci and 4 per cent showed small frankly invasive but occult carcinomas.

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Discussion

Anthony F. Anderson, Edinburgh, Scotland, U.K.: Bickenbach does not seem to talk about removed uteri at all. I would ask, from his last sentence, why make the task difficult when a punch biopsy has already shown invasive cancer? We have seen, and published, this being done by two different hospitals and as the second biopsy was negative the patient went untreated for four years, before being diagnosed anew, as Stage II cancer.

Campos also does not talk about removed uteri and does not answer the question. I believe we cannot use the final diagnosis of carcinoma *in situ* until the uterus is removed. But we have to act on the biopsy report. Therefore the "percentage" answer required in this question depends on the size of the biopsy and will vary inversely with it.

Fidler and Boyes again come up with the answer—comprehensive and comprehensible.

Friedrich Bajardi, Graz, Austria: Ring biopsies or cone biopsies, examined in serial sections, have been performed on 158 patients in whom previous small biopsies showed an "atypical epithelium" or a carcinoma *in situ*. In this way we could demonstrate in 30 cases (19 per cent) an early invasive carcinoma. Two of these cases displayed invasion of the carcinoma into lymphatic vessels. In another case penetration into a small vein could be shown.

Approximately the same results had been ob-

TABLE 1. *Results of Serial Histological Examination of the Postoperative Material in 81 Cases with Preliminary Diagnosis of "Carcinoma in Situ" and "Probable Carcinoma in Situ"*

Carcinoma *in situ*	Carcinoma *in situ* partim invasio incipiens	Carcinoma invasivum	Paratypia susp. quoad carcinoma *in situ*	Erosio colli uteri
71	4 = 4.9%	1 = 1.2%	4	1

tained in a former investigation (Bajardi, F. and E. Burghardt: Arch. Gynaekol. **189:** 392, 1957). Among 61 cases of "carcinoma *in situ*" a beginning invasive carcinoma has been found in ten patients, 16 per cent, by performing serial sections.

Hans F. Bettinger, Melbourne, Australia: I have no actual figures to offer, but the three contributions and especially that of the Canadian authors raises again the question, how to regard "microinvasive foci of minute aggregations of cells?" Should such lesions really be reported, classified and treated as ordinary frankly invasive carcinoma?

Jean de Brux, Paris, France: The differences indicated by the authors provide a striking demonstration of the total lack of precise tests for carcinoma *in situ*, and of the purely subjective character of this diagnosis (Bickenbach, for example, refers to "extremely atypical epithelium" and "plainly atypical epithelium"). Furthermore, the same tests for the invasion of the stroma are lacking.

Hence, I believe it would be very useful to call a conference at which pathologists and cytologists would come to agreement on the definition and, especially, the criteria of carcinoma *in situ*. Why not in Vienna in 1961?

Clarice do Amaral Ferreira, Rio de Janeiro, Brazil: Out of one group of 70 patients with suspicion of carcinoma *in situ*, 44 were submitted to serial sections of the cervix. Thirty-six patients had confirmation of the previous diagnosis and in eight cases, invasion of the underlying stroma was found. Of these, four were diagnosed by amputation of the extirpated uterus. This means a percentage of 9.09. (Reference: Dr. Hildegard, in charge of the Department of Cancer, Instituto de Ginecologia.)

Ronald R. Greene, Chicago, Illinois, U.S.A.: The title of these papers should have been modified by a definition of the term "biopsy diagnosis," since it is obvious that multiple biopsies of adequate size would be more likely to pick up areas of invasion than a single tiny biopsy.

Most, but not all, of the biopsies submitted to the laboratory of the Department of Obstetrics and Gynecology of Northwestern University Medical School are of the four-quadrant variety. Each portion of tissue is bisected and the resulting eight fragments are mounted according to quadrants in one block. Sections are prepared from at least four levels in the block.

To determine how many invasive cancers were missed by this technic, Dr. Frank Maher went over the tissues in this laboratory from 138 patients in whom a biopsy diagnosis of carcinoma *in situ* had been made and a subsequent hysterectomy or surgical conization had been done. These surgical specimens had, of course, been serially blocked. Areas of definite invasion were found in five or 3.6 per cent of the subsequent surgical specimens. In three of the five the original biopsies were of adequate-sized four quadrant variety. One was labelled as a three quadrant biopsy and the last was from two areas of the cervix.

It seems obvious that there is a low but definite inherent error in four-quadrant biopsy diagnosis of carcinoma *in situ* and that one is taking a calculated risk in definitive treatment of a patient on the basis of such a diagnosis.

Maria Kawecka, Jadwiga Bylina, Hanna Starkiewicz, Gliwice, Poland: At the Institute of Oncology, Gliwice, Poland, between 1947 and 1954, hysterectomy was performed in every case of diagnosed cervical carcinoma *in situ*. Eighty-one women were operated on during this period. Preliminary diagnosis of carcinoma *in situ* were based upon four-quadrant biopsies. Serial histological sections of the postoperative material show results in Table 1.

Serial histological examination of postoperative material detected definitely invasive growth in one

TABLE 2. *Results of Histological Examination of Material from Conization in 167 Cases of Cervical Carcinoma in Situ*

Carcinoma *in situ*	Microcarcinoma invasivum	Carcinoma *in situ* partim susp. quoad invasionem incip.	Paratypia maioris gradus et casus limatans	Erosio colli uteri
126	5 = 2.9%	7 = 4.2%	25	4

of the 81 women, or in 1.2 per cent of the cases. Invasive microcarcinoma was found in four cases, or in 4.9 per cent.

Since 1954 our approach toward carcinoma *in situ* has become less radical.

We stated that conization is a sufficient procedure, and that follow up for many years of 167 cases of carcinoma *in situ* did not show recurrence of cancer after conization. Serial histological examination of the material from conization was done in every case (Table 2).

Invasive microcarcinoma was found in 2.9 per cent of the cases. In 4.2 per cent of the cases there was suspicion of invasive growth. Five cases of carcinoma incipiens are alive and well at the end of three years.

Of 248 patients diagnosed as carcinoma *in situ*, there have been found 17 cases of early invasion either on serial histological examination of postoperative material, or on the material from conization. This represents 6.8 per cent of all cases and agrees with the results of Fidler and Boyes.

The discrepancy of our "figures" with those of Bickenbach and Soost is probably due to the different interpretation of "early invasion."

Ernst-Helmut Krüger, Halle a.d. Saale, Germany: Obviously, it is presumed here that the original diagnosis of carcinoma *in situ* has been made from a biopsy specimen. We do not feel entitled to make the definite diagnosis "carcinoma *in situ*" from a simple biopsy. In case an increased atypical epithelium is found in a portion of tissue, in our opinion the only correct wording of the diagnosis should read: "One deals here with a so-called carcinoma *in situ* or with the marginal regions of an invasive carcinoma." In this we agree with Bickenbach and Soost. In case the carcinoma *in situ* has been diagnosed from a simple biopsy, according to experience, there is already an invasive carcinoma present at other sites on the remaining epithelium in 30 per cent of the cases. From 460 biopsies performed in order to clarify this suspicion we were able in only 36 per cent of the cases to confirm the diagnosis of a malignancy.

Hans Limburg, Homburg, Saar, Germany: Unfortunately the diagnosis of beginning invasion is handled quite differently by the various investigators. Therefore, before agreement has been reached concerning uniform handling of the matter, all reports must be accepted with caution. The subsequent finding of an invasion in another site, in cases with the primary diagnosis of carcinoma *in situ*, is entirely dependent upon the exactitude with which all the diagnostic methods for early detection have been employed. By systematic and cooperative use of cytology, colposcopy, guided biopsy and ring biopsy the percentage of these cases can be kept relatively low.

In a period of 19 years (1936 through 1954) we have found, in addition to 268 cases of carcinoma *in situ*, 45 beginning invasive carcinomas up to an extension of five millimeters in depth. Among 107 patients with the diagnosis of carcinoma *in situ* who underwent repeated examinations, only eight cases (7 per cent) showed a simultaneous invasion at another site in the surgical specimen. Furthermore, in 20 of these cases (19 per cent) nothing was found on extensive serial section examination. Thus, it can be assumed that the entire altered epithelium was removed by the colposcopically guided biopsy. This is not unusual, since one also knows of invasive carcinomas that have been completely removed by biopsy. Therefore, we deem colposcopy in combination with cytology necessary in order to gain an impression as to the site and size of the lesion before the final diagnostic procedure, such as ring biopsy or conization is done. Colposcopy is negative in purely endocervical carcinomas *in situ* with no involvement of the ectocervix. However, involvement of the endocervix alone has been rarely encountered in our material, in only 3 per cent of 403 cases of carcinoma *in situ*.

Claud W. Taylor, Birmingham, England, U.K.: A conization specimen may show early invasion not found in a punch biopsy, because it affords more adequate material for study. The point at issue is, however, how often will serial sections show evidence of invasion not discernible in a routine section from each block of adequate material? That this occurs in one case out of every three, as stated by Campos, must be doubted. The answer to the question "How often?" depends on personal interpretation of "invasion." The two histological stages distinguished by Fidler and Boyes—discrete microinvasion and frank confluent invasion—could well be adopted generally. The finding of these authors that serial sections discover 4 per cent of occult, but frankly invasive, carcinomas seems to be a significant answer to the question under discussion.

Closing Remarks

Werner Bickenbach and Hans-Jürgen Soost: As the discussion has shown, all participants agree that the final diagnosis of marked atypical epithelium (so-called carcinoma *in situ*), cannot be based on an individual biopsy, since the edge of an invasive carcinoma may hide itself behind such a pattern. However, it is striking how the percentages of the detected invasive carcinomas varied among the different authors (from 6 to 30 per cent), if the first diagnosis was based on an individual biopsy. In our opinion, the cause of this variation is the different basic investigative work rather than a different judgment of invasive growth. It might be evident that one finds the percentage rate of secondarily detected invasive carcinomas to be far lower, if the initial diagnosis had been performed by four quadrant biopsy (Greene) or as compared with the approach in which in suspicious cases the

ring-biopsy had been performed together with cytology and colposcopy (Limburg).

The relatively high percentage rate of invasive carcinoma, which we found by consecutive conization, is certainly to be related to the fact that in our material the first punch biopsy had often been done by a practitioner in his office. In the hospital we perform conization in any obscure case. The simple biopsy is carried out merely on obvious carcinomas. Our results are mainly based on the comparison between simple biopsy and electro-conization, because in most cases we treat cervical carcinoma by combined radium-roentgen therapy and we operate on cervical carcinomas of Stage I only exceptionally. In only few cases we perform total hysterectomy after conization. In our opinion, the diagnosis of a so-called carcinoma *in situ* can be verified only by conization. Three cases, reported by us elsewhere in which the initial biopsy had shown a small invasive carcinoma and the following conization had proved nothing atypical, had been treated radically, of course, as true carcinomas by extensive radium-roentgen therapy.

Herbert Fidler and D. A. Boyes: The discussion emphasizes the point that the investigation of suspected carcinoma *in situ* of the cervix is incomplete without serially blocking and step serial sectioning of adequate cervical tissue—in our opinion a wide, cold knife cone biopsy. In answer to Bettinger's question regarding the case with scattered, discrete micro-invasive foci only, we believe the prognosis closely approaches that of purely carcinoma *in situ*, and we are currently recommending conservative management as for carcinoma *in situ*. More information is required regarding this point, but we have not yet experienced any recurrences. In contradistinction, those cases with small, confluent, frankly invasive carcinomas should be classified with Stage I lesions, and of the 20 such cases cited, two have died of their disease.

The histologic diagnosis of early invasion, as pointed out by de Brux and Taylor, is certainly subject to differences in interpretation and there is need for clearer and more generally accepted definitions.

Nomenclature of the Atypical Epithelium

FRIEDRICH BAJARDI
Graz, Austria

IN our opinion this subject comprises two basic questions:

1. Which is the most useful gradation of the various forms of the pathological surface epithelium of the uterine cervix?
2. Which morphological criteria shall be used for distribution of various kinds of epithelium into individual groups of a certain selected nomenclature?

In regard to the usefulness of a nomenclature, it is desirable that it be based upon cytological criteria as they appear in the histological section. Thereby, correlation with the findings of exfoliative cytology will be enhanced. The degree of cellular changes permits certain prognostic conclusions. A decision as to the necessity of follow up examinations will depend upon the extent of these changes. As a matter of fact, most of the classifications of the pathological squamous epithelium have been developed according to these principles.[2-4, 6, 7, 9]

The prognostically less important forms of growth of surface epithelium, e.g., bud formation, ingrowth into cervical glands, etc., may, if at all, be considered in a supplementary nomenclature.

It is of special importance that the nomenclature, including the prognostical implications contained in it, be discussed between histologists and clinicians. An agreement on an international basis would be extremely desirable because of the increasing importance of the whole area.

We, ourselves, use for our histological examinations the nomenclature as recommended by Glatthaar and Mueller.[4] The reason for selecting this scheme is that in our opinion the division of the pathological squamous epithelium into four classes cor-

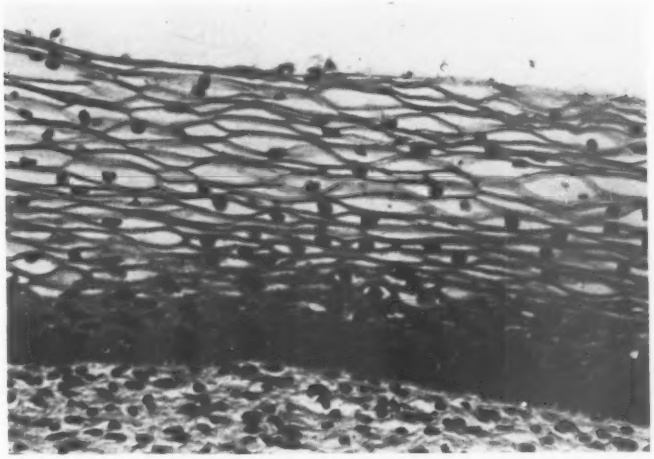

FIG. 1. Normal epithelium (Class I).

responds better to the needs of the clinician in prognostical regard than the division into three subgroups recently proposed by Mueller.[8] We want to express by the diagnosis "carcinoma *in situ*" or "surface carcinoma" that we think a regression of these alterations is almost impossible but a progression towards an invasive carcinoma may well occur. In the case of "atypical epithelium" we make our prognosis in a similar way, however, a regression towards the benign epithelium is considered possible, if only for a few cases. The continuous observation of some of such cases as recently done in the University Women's Hospital in Graz by Burghardt[1] seems to confirm this assumption, being in agreement with the results of Haefeli.[5] When an "unquiet epithelium" is diagnosed we restrict ourselves to control examinations in three to six months intervals, since a maldifferentiation towards atypical epithelium or even surface carcinoma is a relatively rare occurrence (within one year in 20 per

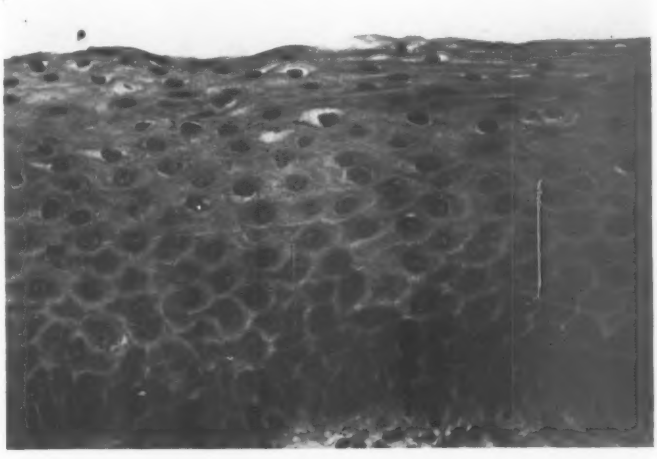

FIG. 2. Abnormal epithelium (Class II).

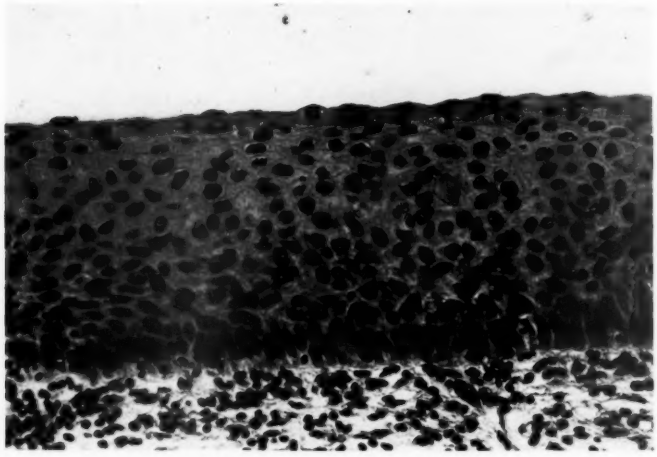

FIG. 3. "Unquiet epithelium" (Class III).

cent of the cases, Haefeli).

The behavior of the "abnormal epithelium" is, according to our experiences, prognostically completely favorable. Its delimitation from the normal squamous epithelium we deem desirable for the following reasons: Colposcopically suspicious findings as well as the frequent pathological findings of the iodine test have their cause, oftentimes, in these minimal epithelial changes.

As a separate group we finally have the "undifferentiated regenerative epithelium." In this group follow up examinations are also indicated, though at longer time intervals, because there is a probability that the "juvenile" and largely undifferentiated cells may differentiate in the wrong direction, i.e., towards any form of pathological epithelium.[4]

Examples of the various epithelia are given in Figures 1 to 5, which correspond approximately to the morphological criteria as given by Glatthaar:[4] The normal epi-

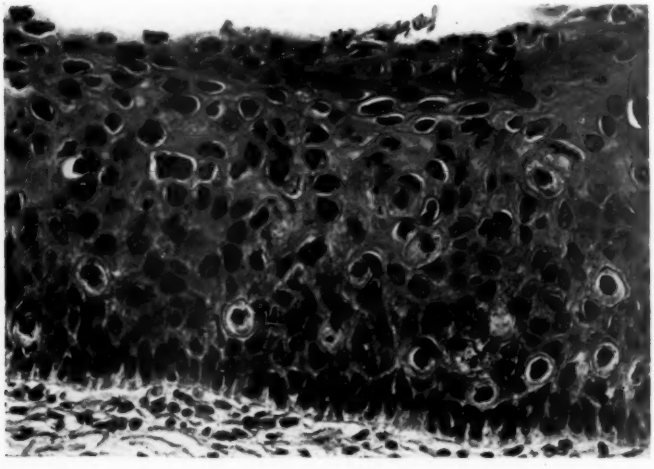

FIG. 4. Atypical epithelium (Class IV).

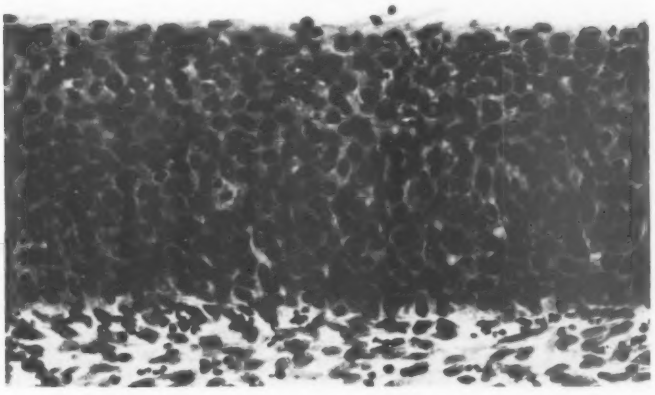

FIG. 5. Carcinoma *in situ* (Class V).

thelium is characterized by the glycogen-containing cells of the stratum lucidum, whereas the cells of the "abnormal epithelium" are free of glycogen and its stratum spinosum is broadened. In the "unquiet epithelium" the changes are more prominent in the intermediate and deeper cellular layers. There is a marked increase of cytological atypia and increase of mitoses in the "atypical epithelium." The "surface carcinoma" has its place at the end of the series. The largely increased number of cells, the numerous mitoses and the complete lack of any maturation towards the surface are unmistakable features of this lesion.

Finally, the undifferentiated regenerative epithelium exists in only a few layers. Its cells almost correspond to basal cells. Nearly always one finds a cellular polymorphia and hyperchromasia of the nuclei.

Linguistic difficulties are the main hindrance for the very desirable international agreement on histological nomenclature.

FIG. 6. Undifferentiated regenerative epithelium (Class R).

Considering, however, how easily the cytological nomenclature as recommended by Papanicolaou has been internationally accepted without resistance, there should also be an international agreement in regard to questions of histological nomenclature within reach. The proposal could be made, as in cytology, to classify the histological pictures in groups. Accordingly, Class I would be normal epithelium, Class II "abnormal epithelium," Class III "unquiet epithelium," Class IV "atypical epithelium" and Class V "carcinoma *in situ*." The "undifferentiated regenerative epithelium" could be classified in a special group "R." A similar classification into groups has recently been recommended by Mueller.[8]

In our opinion, an undebatable advantage of the proposed classification is the fact that the cytological alterations as they appear in the histological section correspond to a certain degree to the respective classes in exfoliative cytology.

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WERNER BICKENBACH AND HANS-JÜRGEN SOOST
Munich, Germany

IN our opinion the term carcinoma should be only used in conformity with the principles of general pathologic diagnosis. The most important characteristic of cervical carcinoma is the infiltrative growth. The nomination of "extremely atypical epithelium" ought to be preferred to carcinoma *in situ* or surface carcinoma.

The word "carcinoma *in situ*" means a prospective evaluation, but as a diagnosis it is not correct, because the extremely atypical epithelium (carcinoma *in situ*) is *not carcinoma,* neither in clinical aspect nor in pathological anatomical conception, as it is acknowledged generally today. We only know that in a certain percentage of the cases it may become invasive and malignant. Definite criteria as to which cases will become invasive do not exist.

Thus, we should not be influenced by the term "carcinoma" *in situ*. According to our experience it misleads numerous physicians to too far reaching operations or radiation therapy.

Otherwise, we follow roughly the classification of Hinselmann. We differentiate:

1. the normal or original epithelium;
2. the plain atypical epithelium;
3. the extremely atypical epithelium, which differs from (2) by more intense polymorphism of the cells and nuclei and more frequent atypical mitosis; and
4. the carcinoma, the most essential characteristic of which is the definite infiltrative growth. The so-called microcarcinoma is not used by us either in diagnosis or in clinical therapy.

Ingrowing into preformed luminae of glands happens in groups 2 and 3. In these cases invasive growth can be simulated when the epithelium of the glands has dis-

appeared. In this case it is necessary for differentiation to perform series sections with or without turning the paraffin block.

We do not place too much importance on the perforation of the epithelium through the so-called basal membrane. Indefinite structures of the nuclei of the basal membrane are also found in atypical tissue unrelated to carcinoma.

The conception of premalignancy is not used by us, because the observed changes are mostly not the preliminary state of a real carcinoma. The conception of premalignancy therefore is not considered with marked atypical epithelium. We confine ourselves to the four groups mentioned above and avoid further differentiation because of the danger of not having an adequate survey. We rather think that any existing atypical tissue structures and the carcinomas can be classified into the groups mentioned above.

JEAN DE BRUX AND H. WENNER-MANGEN
Paris, France

OF first importance is to define the term *atypia*, for the many interpretations given it by the different authors have led to a total confusion concerning the lesions thus designated.

Etymologically, the term atypia refers to a process deviating from the normal type and resulting in the formation of a tissue different from the physiological regenerations, and likewise distinct from the classical cancers by their absence of invasion.

Unfortunately, the term atypia employed in the cytological sense has become one of the criteria of an element's malignancy, and, too often, synonymous with the concept of cancer.

Since Hinselmann introduced into cervical pathology the histological term of "atypical epithelium," this designation has acquired a clinical significance corresponding histologically to a whole series of very diverse lesions, ranging from simple hyperkeratosis to—and or not including, depending on the author—carcinoma *in situ*. From this arises the extraordinary confusion of interpretations in the presence of a single term employed for such a diversity of lesions.

Some authors (Mestwerdt, Mikulicz-Radecki, Bruntsch, Buengeler, Dontenwill, Martius) reject the term "carcinoma *in situ*," which is heavily loaded with consequences not only theoretical, but also clinical and therapeutical.

Similarly, Held and Bickenbach in 1952, proposed the term "non-invasive atypical epithelium." These authors, opposed to the term "carcinoma *in situ*" believe that two-thirds of the lesions labelled "surface carcinoma" are reversible.

Dyroff and Feyrter emphasize the limited significance of the term "carcinoma *in situ*," meaning virtual or potential malignancy, to such an extent that Daufman in 1956, in order to put an end to the dispute, proposed to consider the expression "aggravated atypical epithelium" as synonymous with "carcinoma *in situ*."

We are vigorously opposed to this terminology, so long as there exist no universally accepted architectural or cellular criteria permitting the affirmation of a diagnosis of intra-epithelial carcinoma, i.e., a lesion still developing on the surface, but potentially invasive.

We, of course, understand the notion of aggravated atypical epithelium, but we neither employ nor accept the term, and we cannot consider it as a synonym for carcinoma *in situ*. We cite as an example certain very active undifferentiated metaplasias, localized or extensive, with numer-

TABLE 1

Hinselmann, Mestwerdt	Plain atypical epithelium I and II	Moderately aggravated atypical epithelium I - II—II - IV.		Aggravated atypical epithelium III and IV
Wespi, Müller	Plain atypical epithelium	Hyperactive epithelium—"restless" (unruhige) epithelium (Deelmann, Müller)	Non-cancerous aggravated epithelium	Cancerous aggravated epithelium
Held, Miculicz-Radecki	Plain atypical epithelium	Hyperactive epithelium		Non-invasive aggravated atypical epithelium
Askanazy, Glatthaar	Abnormal epithelium (Askanazy)	Hyperactive epithelium	Atypical epithelium	Surface cancer
R. Meyer, Treite, Limburg	Non-suspect epithelium	Hyperactive epithelium	Suspect epithelium	Surface cancer
Galvin, Te Linde, Jones	Basal-cell hyperplasia, Stage I	Stage II	Stage III	Cancer *in situ*
Jordan, Bader, Day	Minor atypia		Major atypia	Cancer *in situ*
Reagan, Hicks, Scott	Atypical hyperplasia, slight degree	Moderate degree	Marked degree	Cancer *in situ*
Palmer, De Brux	Regular dysplasia	Regular dysplasia with basal hyperactivity	Irregular dysplasia	Intra-epithelial carcinoma

ous mitoses and anomalies, for which it is very difficult to avoid the diagnosis of carcinoma, but which nevertheless heal, either spontaneously by progressive epidermoid differentiation and maturation, or by localized excision with subsequent formation of abnormal epithelium.

We have, therefore, banished from our vocabulary the expression "atypical epithelium," the absence of sufficient inherent precision in this term leaving the door open to too many interpretations and abuses.

We prefer the term "dysplasia" which, with R. Palmer, we have employed for the past eight years.

The dysplasia is a zone of surface epithelium whose structure, independent of any inflammatory or hormonal action, is lastingly different from that of a normal epithelium. It is iodine-negative to the Schiller test, and its outline is distinct.

Under this term we recognize three aspects:

(a) *Regular dysplasias,* distinguished by the persistence of a regular architecture, with some anomalies in relation either to slight disturbances of cellular maturation, or to the hyperactivity of the basal cells.

(b) *Irregular dysplasias,* characterized by the more or less complete loss of stratification, owing to a delayed cellular differentiation, may be severe disturbances of the maturation.

(c) *Intra-epithelial* carcinoma, characterized by:

- the undifferentiation or uncertain differentiation of the cells of the entire epithelium;
- the crowding and multiplicity of the cells;
- the total or nearly-total immaturity;
- the nuclear anomalies, the mitoses, and the inversion of the N/C ratio.

Nevertheless, an important problem still remains; that of differentiating between the intra-epithelial carcinoma and the active undifferentiated metaplasia, which in its most severe form is manifested during pregnancy, and which, in spite of its strong resemblance to a malignant lesion, is not a cancer, since it heals—peculiarities which have led us to adopt for such lesions the term "carcinoma *in situ*-like." The differential diagnosis between these two lesions (intra-epithelial carcinoma and active undifferentiated metaplasia) is based on a study of the smears: The active undifferentiated metaplasia is characterized by a discrepancy between its histological aspect, which appears malignant, and its exfoliated cells, whose nuclear morphology in no way resembles that of cancerous cells.

Discussion

Jean Berger, Basel, Switzerland: At the present time there are a number of nomenclatures used for changes of the squamous epithelium of the cervix uteri, which basically express the same meaning in different terms. If one looks at an assembly in a chronological sequence, as de Brux did, one will recognize that at the present time the term "atypical" is mainly confined to carcinomatous, irreversibly changed tissues. This can be traced back to the pathologist Askanazy who, twenty years ago, wanted the term "atypia" and "atypical" restricted for carcinomatous tissues. Even though we still adhere to the classification given by Hinselmann (for want of a better one), the classifications given by de Brux and Palmer seem very valuable.

However, all classifications are missing a prognostic evaluation, so important for the clinician. Thus, the question is posed: What should be done with an "irregular dysplasia"? Will an amputation of the cervix suffice or, according to the age of the patient, should a complete hysterectomy be performed? Therefore, we have introduced a classification expressed by figures which are analogous to Hinselmann's classification which is in use at the Basel University Women's Hospital.

More modern classifications may arise from histochemical, fluorescence-microscopical and perhaps also electron-microscopical examinations. It remains important for the clinician that not only are new terms invented, but that there is some concrete statement as to the prognosis of the lesion.

Hans F. Bettinger, Melbourne, Australia: Bajardi is quite right when he states that the main hurdles

TABLE 1

Numerical classification (Basel University Women's Hospital)	Histological changes	Advice to the clinician
−3 −2	Abnormal epithelium	Observation and cytological control
−1	Increased atypia	Confirmation of diagnosis. Vaginal cytology, biopsy, repeat cytology and curettage.
0	More marked intra-epithelial changes	Reassurance of whether or not an invasion is present. In any case, immediate therapy: amputation of cervix or simple total hysterectomy.
+1	Definite invasive carcinoma	Wertheim operation or Roentgen-radium irradiation.

with regard to nomenclature of the atypical epithelium are linguistic difficulties. A literal translation of the German terms into English does not convey their proper meaning and to have three classes between the normal epithelium and the carcinoma *in situ* is only adding to the difficulties of demarcation.

Many will share the view of Bickenbach and Soost that a carcinoma *in situ* is really not a carcinoma in the ordinary sense, but I am afraid it is now too late to eradicate this term. We prefer with de Brux to call the wide range of lesions which lie between normal epithelium and carcinoma *in situ* "dysplasias," and are in general in agreement with his arguments.

Jorge Campos R. de C., Lima, Peru: It is highly desirable to obtain a general agreement between cytologists, histologists and clinicians with regard to the nomenclature of the atypical cervical epithelium. Nevertheless, the objective is difficult to reach at the present time because of the deficiency in the fundamental knowledge regarding the evolution and prognosis of carcinoma *in situ*. Since we are not sure that carcinoma *in situ* is really a malignant condition, this term will be the theme of our objection.

If we follow the classic criterion, the word "cancer" must be used only to designate invasive tumors with metastatic possibilities and therefore, the terms "carcinoma *in situ*," "non-invasive cancer," "intraepithelial cancer" are incorrect.

However, if we follow a less classic but more cytological criterion and accept that the essential point in the morphologic diagnosis of cancer is in distinguishing the normal cells from those that are cancerous, then the objections to the term "cancer *in situ*" disappear because the morphologic differences between each type of cell are supported by variances in the physiological behavior and chemical composition of the cells, and the cancer *in situ* cells can be estimated as cells that individually are neoplasms.

Judging from the facts, invasion and metastasis are important phenomena but not fundamental for the concept of malignancy; they are a consequence of alterations in the behavior and cellular physiology which can be estimated by the histologists and the cytologists as morphologic changes. For that reason, the criterion of Bajardi seems acceptable with regard to the fact that any classification of atypical cervical epithelium should be supported by a cytological criterion, which should have the advantage of permitting, if possible, a correlation between histology and exfoliative cytology.

The term "dysplasia" seems to be convenient; in referring to cases which have been called "hyperactive epithelium" but not carcinoma *in situ* (Wespi and Müller; Held, Mikulicz-Radecki, Askanazy ad Glatthaar, Meyer, Treite and Limburg) or as "atypical epithelium" (Hinselmann and Mestwerdt, Askanazy and Glatthaar, Bajardi). The word "atypical" is very often associated with cancer; "dysplasia," on the contrary, has the advantage of not prejudging the future epithelial behavior.

I disagree with de Brux and Henriette Wenner-Mangen when they state that there is no universally accepted criterion regarding the architecture of cancer *in situ*. I think that Figure 5 of Bajardi should be accepted universally as a good example of carcinoma *in situ*. The objections against the term are not for the purpose of disagreeing with the histologic structure but because of ignorance concerning the future evolution of this lesion.

Wolfgang Korte, Bonn, Germany: If one follows the known histological definitions, then the atypical pattern of cells, tissues and tumors is a true criterion of malignant tumors.

No colposcopist, cytologist or histologist will consider as malignant the "simple atypical epithelium" of Hinselmann, as quoted by Bickenbach and Soost.

One knows from experience that some commonly used medical terms can hardly be eliminated, even if they are grammatically illogical or etymologically bad or incorrectly composed.

If one begins with the term "normal epithelium" one has to name the not normal epithelium "abnormal" (Latin) or "anamalous" (Greek). If one wants to maintain the cytological term "atypical epithelium," then one would have to designate the normal epithelium as "typical." I propose to use the terms "normal" and "not-normal" epithelium up to the time of an international agreement (perhaps at the next International Cytology Congress in Vienna in 1961).

The "not normal epithelium" then can be divided into "not-normal benign epithelium" and "not-normal malignant epithelium." Clinicians, pathologists, cytologists, histologists and colposcopists would understand this terminology. Each could add an appendix in parentheses according to the common usage in his school or geographical area. For instance: "Not-normal benign epithelium (simple atypical epithelium of Hinselmann)."

Ernst-Helmut Krüger, Halle/Saale, Germany: I believe that a classification of the histological patterns according to the cytological group classification, as proposed by Bajardi, is worth discussing. In this case I would like to see the abnormal and "unquiet" epithelium included in Class II, or the "unquiet" epithelium in Class III. In this latter, somewhat suspicious, group, I would also like to see included the so-called carcinoma *in situ*, since, at the present time its obligatory addition to Class IV would mean to us a too close association with carcinoma. The undifferentiated regenerative epithelium also would belong in this group of suspicious epithelial conditions. We agree completely with the statements of Bickenbach and Soost. We also use the original Hinselmann's nomenclature with four groups. For a definite diagnosis a serial section examination of the entire suspicious tissue is indispensable. We do not say "carcinoma *in situ*" but restrictively "so-called carcinoma *in situ*." Probably the best definition of its character is given by the designation of Held—"not invasive

atypical epithelium" or "not yet infiltrative carcinomatous epithelium." A differentiation between the so-called carcinoma *in situ* and the active undifferentiated metaplasia is for us also impossible. This question was brought up by Henriette Wenner-Mangen and de Brux. We do not use the term "dysplasia." A uniform international nomenclature of the atypical cervical epithelium would be highly desirable. Is it not already contained in the old Hinselmann's nomenclature? I believe so.

Otakar Nyklíček, Náchod, Czechoslovakia: The discrepancy in opinions concerning the pathological differentiation of the cervical epithelium also appears in the reports of the three main authors. Even for an enumeration of suggested nomenclatures a great deal of room would be necessary. Every author employs his own nomenclature.

This lack of uniformity in histopathological nomenclature is the reason for considerable difficulty among the gynecologists, and for this reason even opinions concerning therapy are often different.

So that order may be reached, a very necessary and useful agreement has taken place in Czechoslovakia between histologists, gynecologists and oncologists. After very thorough preparation, a panel conference of the above three societies was held in April, 1959, at which time conclusions were reached for a uniform nomenclature which would be obligatory in the future for all hospitals in Czechoslovakia. I take the liberty of reporting here, in an abbreviated form, the conclusions of this conference (published in "Čs. Gynekologie," 24:7, 1959). The nomenclature group directed by Professor Pazourek suggested these forms of nomenclature for epithelium when pertaining to the cervix: I. Abnormal epithelium; II. Atypical epithelium; III. Preinvasive carcinoma; IV. Invasive carcinoma.

I. Abnormal epithelium: This is composed of those epithelial changes whose evolution to carcinoma has not been definitely proven, and which arise predominantly through adaptive processes of various pathological states of the cervix, or by a derangement of differentiation due to various causes. It is taken for granted that such an epithelium is differentiated from the normal epithelium, or remains stationary, if no other factors intervene. The most important morphological characteristics are as follows: The stratification is not greatly disturbed. The stratum lucidum is very seldom present, and the stratum spinosum is more widely spread. A parakeratosis may be formed on the surface. A mild polymorphism of cells and an increase in the number of mitoses is found. The epithelium grows against the base in the form of shallow processes and sometimes may penetrate the lumen of the glands.

II. Atypical epithelium: The differentiation of the cells is lessened, so that these show the features of basal elements. The stratification of the epithelium disappears completely. The polarity of cells changes, their nuclei occupy a position perpendicular to the basal membrane. The cyanophilia and nuclear-cytoplasmic ratio change. These are changes whose relation to the evolution of carcinoma has been proved, but they still can be reversible, stationary, or show a tendency to further anaplasia, even to preinvasive carcinoma.

III. Carcinoma in situ: A complete loss of cellular differentiation, so that cytomorphologically the epithelial layer shows all the characteristics of carcinoma. Neither infiltrative growth nor invasion into vessels can be present. A diagnosis of carcinoma *in situ* cannot be given before careful study of serial sections of the surgical specimen has been performed.

The uniform histopathological nomenclature has also quite substantially unified the opinions concerning the therapy of these important changes in the cervical epithelium.

Rodolfo Sammartino, Buenos Aires, Argentina: The process of pathological proliferation is naturally very complex in the cervical epithelium. Therefore, there is no justification for the tendency to simplify it artificially. On the contrary, we must define and classify as exactly as possible all the characteristic pictures of pathological growth, with the object of appraising the real relationship of each lesion with carcinoma. For this purpose we use the following classification which includes the principal changes:

Group I: Abnormal Squamous Epithelium (clinically benign)
- Aglycogenous (dark, non-vacuolated cytoplasm)
- Hypertrophy (diffuse thickening without alterations of the layers)
- Basal hyperplasia (stratification of columnar regular mature cells)
- Keratosis, hyperkeratosis, parakeratosis
- Epidermization by erosion and ectropion
- Squamous metaplasia of the mucous epithelium (by proliferation of the basal reserve cells)
- Regeneration, by inflammation (narrow, immature epithelium)

Group II: Restless Squamous Epithelium (suspicious for malignancy)

Group III: Atypical Squamous Epithelium (possibly or definitely malignant)
- Non-invasive (intraepithelial carcinoma or carcinoma *in situ*)
- Invasive (microcarcinoma, macrocarcinoma)

We avoid as much as possible the use of ambiguous terms, such as: dystrophia, dysplasia, leukoplakia, undifferentiated hyperplasia, anaplasia, etc., and also the term "carcinoma" for non-invasive atypical epithelium.

Claud W. Taylor, Birmingham, England, U.K.: Nomenclature to describe adequately a series of progressive changes within an epithelium is diffi-

cult to devise. The term "atypical epithelium" should be broad in its application; as pointed out by de Brux and Henriette Wenner-Mangen, this word has been given undue significance in cytological interpretation. The recommendation of these authors that "dysplasia" with sub-classifications should be adopted has much to commend it. However, as re-emphasized by Bickenbach and Soost, the use of the word carcinoma for an *in situ* or intra-epithelial lesion can be condemned within the principles of general pathologic diagnosis; neverthless, it seems inevitable that this usage will continue. Any nomenclature proposing numerous sub-classifications only leads to confusion.

Wolfgang Walz, Heidenheim a.d. Brenz, Germany: It would be very desirable if an agreement could be reached among the Members of the International Academy as to the nomenclature of the "atypical epithelium." The classification for the various forms of the atypical epithelium as given by Bajardi corresponds to the original School of Zürich nomenclature (Glatthaar, Müller, Wespi) which fulfills, in our opinion, all requirements. The figures given by Bajardi very strikingly characterize the histological criteria. It does not seem advisable to me to replace the names, e.g., "unruhiges Epithelium," with a numerical classification, e.g., Class I, etc., since only confusion will arise from this; for example as with the Hinselmann classification. Moreover, a nomenclature of this kind is already reserved for cytological reports (Papanicolaou, Class I to Class V). The scruples of de Brux and Henriette Wenner-Mangen against the term "atypical" are understandable since it is not exact in the pathological sense. Thus, it would be advisable to recommend the introduction of "dysplasia" instead of "atypical" and transfer this to the Zürich nomenclature.

If one bases the designations for the morphological changes upon the Figures of Bajardi, the following terms could be proposed:

Fig. 1. Normal squamous epithelium.
Fig. 2. Abnormal epithelium.
Fig. 3. Regular dysplasia.
Fig. 4. Irregular dysplasia.
Fig. 5. Carcinoma *in situ*.

All of the remaining histological changes of the squamous epithelium are changes of regenerative epithelia, which either arise as regenerative epithelium from the margins of the squamous epithelium or by so-called indirect metaplasia. We have to add also the changes caused by inflammations (e.g., inflammatorily irritated epithelium). Certainly a dysplasia or a carcinoma *in situ* may arise from these inflammatory lesions, but they can still be distinguished histologically from the true dysplasias and they are not in the histological sense changes "sui generis."

Closing Remarks

Werner Bickenbach and Hans-Jürgen Soost: In our report we wanted to emphasize that the markedly atypical epithelium (carcinoma *in situ*) in its clinical importance has nothing to do with the true carcinoma. This fact is generally acknowledged. Therefore, one may ask, why is one not consistent as far as nomenclature is concerned, avoiding the term "carcinoma"? This term often misleads one to more extensive and unnecessary therapeutic management. The true carcinoma requires extensive surgical and/or irradiative management, even if it is "microinvasive," but the so-called carcinoma *in situ* really does not need such extensive therapy.

There are existing numerous terms, primarily meaning the same thing, for these epithelial atypia which are stages between normal epithelium and invasive carcinoma. A differentiation between "abnormal" and "atypical" is grammatically unfortunate as Korte rightly emphasized, since both terms are not essentially different in their significance. But we also believe that the designations "not normal, benign" and "not normal, malignant" are inadequate, since there is a prospective interpretation in these terms. As long as we do not know of any certain criteria which permit a prospective interpretation, it seems better in our opinion to use merely the truly descriptive nomenclature. It should be simple and should not contain too many classes. In our opinion the nomenclature of Hinselmann is still today a good basis for the terminology. Whether one speaks of "dysplastic" or "simple atypical," as proposed by de Brux, seems to us of less importance.

Different authors proposed the discussion of these questions at the next International Congress of Exfoliative Cytology in Vienna in 1961. We think that very advisable. One should, however, allow not only pathologists and cytologists to speak, but also clinicians.

This must be considered further; the term "atypical" right now still has different meanings depending on whether it is used in colposcopic, cytologic, or histologic findings. According to the differing accuracy of the examination methods, differing therapeutic conclusions result.

The Atypical Non-Invasive Zones Around Invasive Cervical Carcinoma

FRIEDRICH BAJARDI

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RESEARCH work on premalignant and early malignant lesions of the cervix invariably leads to giving increased attention to changes of the epithelium in the vicinity of invasive carcinomas of the cervix. With great probability it may be assumed that such localized changes represent pre- and early stages of invasive tumors in their biological behavior. The knowledge of their histomorphology will thus also facilitate the prognostic evaluation of a pathological epithelium situated beyond the area of invasive carcinoma. That is to say, from morphological analogies we may also draw, with some precaution, comparative conclusions with regard to a similar biological behavior of the epithelium. This applies both to changes in the epithelium bordering invasive tumors and to those which can be found in isolated positions.

On the other hand, we find certain difficulties in distinguishing premalignant and early malignant lesions near the border of the invasive tumors.

No strict rules exist in this respect, as the author's investigations proved, and as was especially described in the extensive work of Schottlaender and Kermauner. In other words, it is by no means always the case that an invasive carcinoma is surrounded by a surface epithelium which can be defined as carcinomatous from a morphological point of view. On the contrary, quite different forms of pathological epithelium were observed in the vicinity of tumors, and in some rare cases we could even prove an almost normal epithelium. Above all, this finding has caused us to be cautious in the evaluation of pathological epithelium, and to assume a mere premalignant lesion rather than to give the diagnosis of carcinomatous epithelium partly at least on the grounds of its being located near an invasive tumor. We believe, however, that such is the practice in some quarters, and that this is also the cause for occasionally stating a diagnosis of carcinoma *in situ* on a somewhat arbitrary basis.

The pictures shown herein all depict border regions of invasive cancer of the cervix. The epithelium in Figure 1 was defined as carcinomatous epithelium; in the case shown in Figure 2, we could, however, not decide upon this diagnosis. Ad-

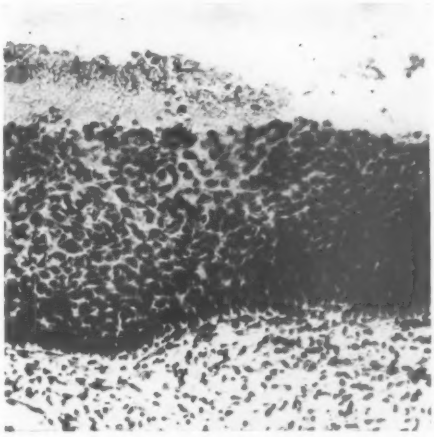

Fig. 1. Carcinomatous surface epithelium located near an invasive carcinoma.

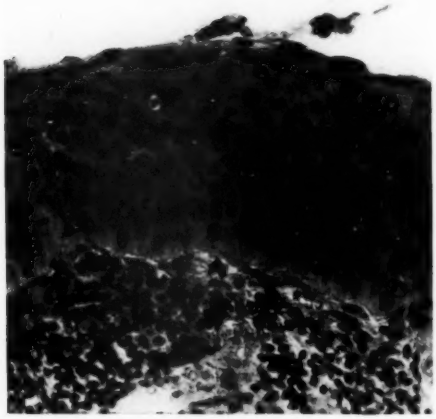

Fig. 2. "Unquiet epithelium" located near an invasive carcinoma.

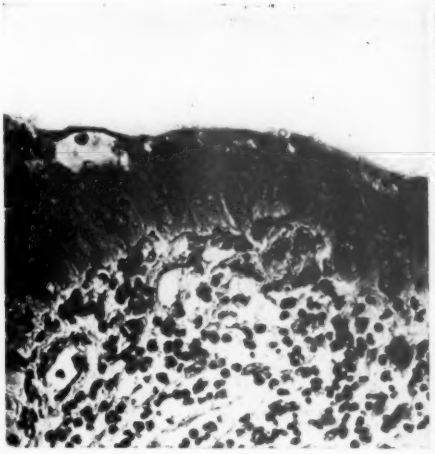

Fig. 4. Carcinomatous surface epithelium located near an invasive adenocarcinoma.

mittedly, the number of cells is considerably larger than is general, and the nuclei are moderately polymorphous in appearance. On the other hand, a distinct maturing of cells, with increasing intensity towards the surface, also takes place. We have termed this change "unquiet epithelium." Finally, Figure 3 shows a relatively thin squamous epithelium showing the picture of an atypical basal hyperplasia. Also in this case the diagnosis of carcinomatous epithelium did not seem justifiable to us.

Figures 4 and 5, on the other hand, demonstrate the fact that the above diagnosis is, indeed, in some cases facilitated or even only made possible by an invasive tumor which is situated close by. Figure 4 shows the carcinomatous epithelium at the border of an adenocarcinoma of the cervix. One would doubtless face certain difficulties regarding the diagnosis, if such an epithelium should be found isolated, i.e., without

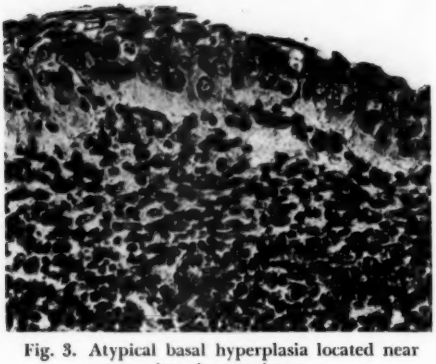

Fig. 3. Atypical basal hyperplasia located near an invasive carcinoma.

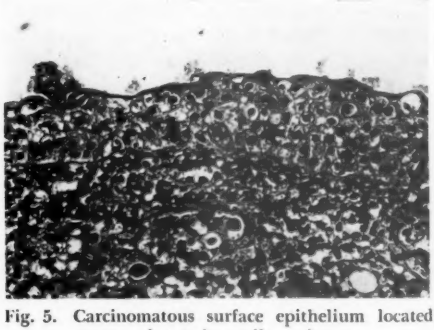

Fig. 5. Carcinomatous surface epithelium located near a signet-ring cell carcinoma.

any relation to an invasive adenocarcinoma. Figure 5 shows the surface epithelium which in its appearance reminds one to a certain extent of an "undifferentiated regenerating epithelium," or of an "unquiet squamous epithelium." Following the establishment of an epithelium morphologically very much similar to the above, localized in the stroma of the cervix, and belonging to an invasive signet-ring carcinoma, we were led here to the diagnosis of also a carcinomatous surface epithelium.

CARLO SIRTORI

Milano, Italy

IN the epithelium surrounding the carcinoma two pictures may be observed: (1) a progressive transformation from normal to malignant epithelium or (2) a sudden transition from normal to malignant epithelium. The peritumoral epithelium is really worth studying, because of the variety of its pathological proliferative pictures, from basal hyperplasia to carcinoma *in situ*.

Discussion

Jean Berger, Basel, Switzerland: There is a wide variety of tissue changes found in the surroundings of a carcinoma. Bajardi shows photographs in which so-called "surface carcinoma" and "increased atypia" may be found. In our cases we, too, have been attentive to the surroundings of the sites of invasive lesions, and we could see that in the majority of the cases atypical epithelium is found in the neighborhood of the invasive carcinomas. In single cases, however, we have been dealing with carcinomatous protrusions side by side with normal squamous epithelium. This demonstrates that there are no gradual transitions from normal to abnormal to atypical and finally to carcinomatous epithelium.

Jean de Brux, Paris, France: From a speculative viewpoint, the atypical zones around an invasive carcinoma are very important. They sometimes (though not always) present, isolated or in groups, the range of lesions from the metaplastic epithelium to the carcinoma, from the regular dysplasias to those more and more irregular. But this succession of lesions is not necessarily progressive toward the zone of the invasive carcinoma. Thus, one may see very suspect zones followed by an only slightly atypical epithelium and then by a zone of greater gravity.

These facts show that the starting-point of the lesions is in the reserve cells. The differentiation of these cells is the "corner-stone" of every lesion. The more this differentiation of an originally multipotent cell is slow in appearing, the more the lesion will be suspicious. But if there is differentiation, and if there is a beginning of stratification, followed by a maturation—even irregular—the lesion cannot be suspicious.

It is only when the differentiation is absent or very uncertain, there is no stratification, and the maturation is very late or absent that one may suspect a cancer.

But the diagnosis should be borne out by a study of the isolated or grouped cells, which should show a marked density of packing, a nuclear hyperplasia with regular chromatin in voluminous clumps, a thickening of the nuclear membrane and some anomalies.

The study of the atypical zones around the invasive cervical carcinomas has led us to the idea of a unity of the process of repair. But we have yet to discover why, at one point, the reserve cell is simply hypertrophied and becomes rapidly differentiated, whereas at another point it is hyperplastic and shows no tendency either toward differentiation or stratification (hyperplasia of the reserve cell). Why, with the same characteristics, only slightly more marked (density, chromatin in clumps, some anomalies), does it tend to invade?

Raimund Krimmenau, Dresden, Germany: Carcinomas either border directly on normal epithelium or on a carcinoma *in situ*, with or without glandular involvement (which itself usually borders directly on normal epithelium), or gradually pass into a carcinoma *in situ*, "unquiet" epithelium (dysplasia), basal hyperactivity, and finally to normal epithelium. From this, however, it cannot be concluded, according to general pathological points of view, that carcinoma develops intermediate stages from the normal epithelium.

The relative areal extension of a carcinoma *in situ* with or without involvement of glands to invasive carcinoma is more on the side of the carcinoma *in situ* (in other words: "extensive carcinoma *in situ* with minimal invasion"). This is the greatest danger of false diagnosis if there is no thorough survey over the entire region (i.e., in wedge biopsies), for in this case with a carcinoma *in situ* or an "unquiet" epithelium you are never certain whether or not it is only the margin of an invasive carcinoma. This uncertainty is generally recognized today. We therefore fully agree with the two main speakers.

Claud W. Taylor, Birmingham, England, U.K.: There seems general agreement that the stratified epithelium of the cervix adjacent to a carcinoma may be normal or show a variety of changes. It is difficult to be sure whether the changes mentioned precede or follow the onset of the carcinoma. Not rarely carcinoma *in situ* is found adjacent to invasive carcinoma; here again, from the study of sections it may be imprudent to decide which lesion came first. It is well to remember that in other parts of the body a surface epithelium can show reactionary changes to an underlying or adjacent carcinoma (e.g., Paget's disease of the nipple) or to non-neoplastic lesions (e.g., ulceration, granulomata). The nature of the relationship of "atypical noninvasive zones around invasive cervical carcinoma" is not known.

Rudolf Ulm, Vienna, Austria: The existence of a noninvasive atypical zone around a cervical carcinoma should not be regarded as the usual occurrence. If such a zone exists, it forms a very instructive object for the study of morphological variations of atypias up to invasive carcinomas. It would be wrong, however, to consider every epithelial atypia on the edge of an invasive cervical carcinoma as being carcinomatous, because not every carcinoma has such a border zone. The atypical epithelium might have existed for a long time and the carcinoma might have developed secondarily in its neighborhood. The atypical noninvasive zone around an invasive carcinoma, therefore, should not be considered as a pre-stage of a cervical carcinoma.

Closing Remarks

Friedrich Bajardi: Theoretically there are various possibilities to explain the existence of atypical, noninvasive zones around an invasive carcinoma:

1. The atypical noninvasive zone has been existing in the form of a carcinoma *in situ*, and the invasive carcinoma has evolved from this noninvasive zone. If this is the case, then the possibility exists that later on in the remaining noninvasive zone, there will also arise invasive tumor growth.

2. The carcinoma does not only grow invasively into the connective tissue, but also superficially towards the normal epithelium, which is displaced and destroyed. The atypical noninvasive zone, in this case, would be a horizontally growing part of the invasive carcinoma. Here too, the invasive potentialities of the noninvasive zone can hardly be doubted.

3. The tumor cells of the invasive section cause a gradual "cancerization" of the adjacent normal squamous epithelium (assimilatory growth). Thus, the surface epithelium is included within the tumor area. Again, potentialities of these cancerized superficial cells to secondary invasion seems probable.

4. The atypical, noninvasive zones arises independently from the invasive carcinoma. Because of their frequency it can be assumed, however, that for the formation of both the invasive tumor and the noninvasive zone, the same type of etiological factors are effective, merely acting in different degrees of intensity. A secondary increase in intensity may also cause the noninvasive zone to develop into an invasive carcinoma.

Thus, we agree with Taylor that it is impossible to decide which has been the primary lesion. In contrast to Ulm, however, we believe, that the atypical noninvasive zones are at least pre-stages of occasional invasive growth.

Carlo Sirtori: Berger admits, as we do, that cancer can be surrounded by transitional areas from normal to malignant. We can presume that carcinogenesis not only affects a rather wide area, but does not begin simultaneously. To the same extent, in the epithelium we con observe invasive carcinoma, a carcinoma *in situ* and a dysplasia. In such cases, as I have already observed in cancers of the oral cavity, relapses can be due to a further carcinogenesis in an area contiguous to the tumor.

Krimmenau, Taylor and Ulm talk about the histogenesis of cancer. I think that the most valuable researches in this field are those of von Haam and Scarpelli (Cancer Res. 15:449, 1955), which reveal that cancer is preceded by a gradual transformation of the epithelium, from hyperplasia to carcinoma *in situ*.

Histochemistry of Carcinoma *In Situ*

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It is a classical statement that malignant lesions of the cervix do not contain glycogen. This is the basis of Schiller's test. Research on glycogen content of carcinoma *in situ* has been negative.[2, 7, 8]

Recently, comparative studies have been made between carcinoma *in situ* and invasive carcinoma[1, 5, 6] without any differences in the glycogen content being found. Only Foraker[3, 4] has occasionally found glycogen in invasive carcinoma, but never in carcinoma *in situ*.

By using the PAS (Hotchkins-MacManus), we have studied 40 biopsies of the cervical epithelium: 12 *in situ* carcinomas, 20 immature invasive carcinomas, and eight mature invasive carcinomas. The results are seen in Table 1.

From these results, we can say that the immature invasive carcinoma has glycogen in 90 per cent of the cases, while the mature invasive carcinoma does not have glycogen, but has mucopolysaccharides in at least 60 per cent of the cases (Fig. 1, 2, 3).

We have previously pointed out[2] that the appearance of glycogen and mucopolysaccharides in the normal squamous epithelium constitutes successive steps of cellular maturation. Therefore, according to our findings, the truly immature carcinoma would be the *in situ* carcinoma, while the invasive carcinoma would always

Fig. 1. Carcinoma *in situ*. PAS, simple reaction, negative. There is neither glycogen nor mucopolysaccharides (900X).

Table 1.

Type of Ca.	No. of cases	PAS without digestion		PAS with amylase		PAS with hyaluronidase	
		Positive	Negative	Positive	Negative	Positive	Negative
In situ	12	0	12	0	12	0	12
Immature invasive	20	18	2	0	20	17	3
Mature invasive	8	6	2	6	2	5	3

Fig. 2. Immature invasive carcinoma. PAS, simple reaction, positive. The amylase digestion causes positive reaction to disappear, so it contains glycogen (900X).

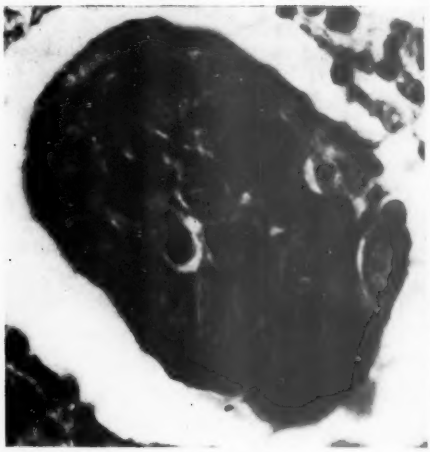

Fig. 3. Mature invasive carcinoma. PAS reaction with amylase digestion. Aspect of a horny globe in which the reaction remains positive showing there is mucopolysaccharide content (900X).

show some degree of maturation, even in cases that appear more immature.

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MANY investigators have applied various histochemical technics to the investigation of carcinoma *in situ,* in more or less sporadic fashion. Since 1950 we have been screening the histochemical field for technics relating to growth or maturation of squamous cells which may apply to this important problem.[1] Dehydrogenase and phosphamidase activity are more evident in proliferating than in maturing squamous mucosal cells. Disulfide groups are found in regions of keratinization. Glycogen is a fairly reliable cytoplasmic index of cell maturation. Sulfhydryl groups are found in all squamous mucosal cells.

However, these histochemical technics, and other special cell study methods, are dependent in general on growth or maturation of the cells. They do not indicate "benignity" or "malignancy" of cells. None of the technics which we have employed have helped to differentiate carcinoma *in*

situ from either dysplasia or invasive carcinoma. This is an interesting field for additional research, and one in which much progress should be possible in years to come, as new cell technics are developed. At present, according to our best existing knowledge, we do not feel that histochemistry has any practical importance in the diagnosis of lesions in patients demanding an immediate decision and management.

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I. Enzymatic Reactions

The enzymes belong to two groups, oxidative and hydrolytic. For enzymatic reactions frozen sections were cut 10 μ thick, put on slides, and mounted in glycerine jelly after staining. Enzymatic reactions were stained with and without hematoxylin counterstain for comparison, except for the phosphamidase reaction which gives nuclear staining. Every reaction was accompanied by controls in which the substrate was omitted or the tissue heated to destroy the enzyme to prove the specificity of the reaction.

The enzymatic reactions studied were dehydrogenase, acid and alkaline phosphatase, phosphamidase, esterase and glucuronidase. For enzymatic reactions formalin or acetone (both at 4° C. for two to four hours) were used as tissue fixatives. That fixative which would cause least destruction of the enzymes was used. The oxidative enzyme *succinic dehydrogenase*,[1] for example, loses much of its enzymatic activity after fixation in formalin. Fixed in acetone, it maintains a great deal of its activity.

Sodium succinate was used as the hydrogen donor, neotetrazolium chloride as the substance to be reduced to diformazan (blue) or monoformazan (reddish), depending on the degree of reduction.

In normal squamous epithelium the tissue showed a reddish reaction in the superficial epithelium, increasing along the basal membrane to dark blue pigment formations. Comparing normal with cancerous tissue there was no significant qualitative difference in reaction intensity: the normal basal cells showed an enzymatic staining quality equal to the cancer cells, but in cancerous tissue there were more undifferentiated cells, so that quantitatively the reaction was stronger. The difference can be demonstrated by simply comparing macroscopically cancerous and normal tissue, both stained with dehydrogenase. The reaction is chemically easily understandable by the reduction of neotetrazolium chloride:

$$RN_4\,CL + H \rightarrow RN_4 \text{ (monoformazan red)} + HCL$$

When the reaction is stronger, two molecules RN_4 form the blue diformazan.

For *alkaline phosphatase*[2] fixation in cold formalin (4° C. for two to four hours) was performed and frozen sections were cut. Sodium α-naphthyl phosphate was used as substrate at *p*H 9.2. This was coupled to the diazonium salt :4-benzoylamino 2:5 dimethoxyaniline: diazo fast blue RR (azo

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TABLE 1.

Substrate	Enzyme	Hydrolyzed product	Diazotate	Dye
O PO_3Na		OH		OH
|	+ alk. phosphatase =	|	+ $R_1N\,N$ =	|
R		R		R
				|
				N
				|
				N
				|
				R_1

Sodium α-naphthyl-phosphate

coupling technic of Pearse). A black precipitate formed in locations of a positive reaction. Comparing normal with cancerous tissue, there was a moderate quantitative increase of the reaction in cancerous tissue. Qualitative cancer cells of carcinoma *in situ* show the same reaction as basal cells. The reaction was complicated by the very strong reaction of the blood vessels, from which diffusion into the surrounding tissue may occur.

The chemical foundation of enzymatic reactions is based on the formation of a phenol coupled to a stable diazo salt (Table 1).

For other enzymes and diazo salts comparable reactions take place.

Azo dyes do not stain the nucleus of the cells, with the exception perhaps in the phosphamidase reactions. In general the best results are obtained by concentration of 1 mg. per cc. for azo dye, and half of this concentration for substrate. *p*H and temperature are of great importance during the reaction. The best temperature for enzyme reaction is usually 37° C., but reactions for enzyme activity are often performed at room temperature 15-20° C. to prevent decomposition of azo dyes.

For *acid phosphatase*[3] fixation in cold formalin (4° C for 2-4 hours) was performed and frozen sections cut. Sodium α-naphthyl phosphate was used as substrate in Michaelis buffer at *p*H 5. This was coupled to the diazotate o-dianisidine (fast blue B salt). A dark blue or black precipitate formed in locations of a positive reaction. Comparing normal with cancerous tissue, there was a moderate quantitative increase of the reaction.

For *phosphamidase*[4] short fixation in cold formalin (4° C. for 2-4 hours) was used and then frozen sections cut. Gomori's substrate p-chloroanilidophosphonic acid was used. A maleate buffer (*p*H 5.6) and manganese ions were used as an activator. With lead nitrate, the corresponding phosphate was treated with dilute ammonium sulfide resulting in lead sulfide as a black precipitate in locations of positive reaction. Comparing with normal tissue, a marked quantitative increase of the reaction in cells of carcinoma *in situ* was found.

For *esterase*[5] fixation in cold formalin (4° C. for 2-4 hours) was performed and frozen sections were cut. α-naphthyl acetate in acetone at *p*H 7.4 was used as a substrate. Fast blue B salt added gives a black precipitate in locations of positive reaction. Compared to normal tissue, a marked quantitative increase of the reaction was noted in tissue of carcinoma *in situ*.

For *glucuronidase*[6] fixation in formalin (4° C. for 2-4 hours) was performed and frozen sections cut. 8-hydroxyquinoline glucuronide in acetone buffer *p*H 5.2 was used as substrate. This was coupled to the diazotate 4-benzoylamino –2:5 dimethoxyaniline (Pearse). An orange-red precipitate was formed in structures containing b-glucuronidase. Comparing with normal

tissue, a marked quantitative increase of the reaction in tissue of carcinoma *in situ* was observed.

Summary of results in enzymatic reactions:

Marked quantitative increase — Glucuronidase reaction
Marked quantitative increase — Esterase reaction
Marked quantitative increase — Phosphamidase reaction
Marked quantitative increase — Acid phosphatase reaction
Quantitative increase — Dehydrogenase reaction
Quantitative increase — Alkaline phosphatase reaction

The enzymatic reactions all show a quantitative increased reaction, especially the first four. Comparing normal and malignant tissue macroscopically after the reaction has taken place, one is able to distinguish malignant tissue by the more intense staining reaction. However, microscopically it is found that this increase is only caused by the larger amount of reacting epithelium in the malignant tissue. In a qualitative sense there is mostly no difference in reaction intensity. The cancer cells show a stronger enzymatic staining quality than the differentiated superficial cells, but an enzymatic staining quality equal to the normal basal cells. In cancerous tissue there are more undifferentiated cells.

Enzymatic reactions stain the cytoplasm. No staining reaction was seen in the nucleus with the exception of the phosphamidase reaction where some nuclear staining was observed.

II. Non-enzymatic Reactions

Cervical material was obtained by punch and cone biopsy of 37 cases of carcinoma *in situ* and invasive cancer. Fifteen to 20 microsections were made from each case so that more than 500 microsections offered more than 40 examples of 12 staining technics, six non-enzymatic and six enzymatic. Every section of malignant tissue was accompanied by a section of normal tissue treated similarly for comparison. A routine hematoxylin-eosin stain was made of every case to compare with the specific stains. For non-enzymatic reactions paraffin sections were used mounted with permount. Counter stain was not used except in the Best stain.

The non-enzymatic reactions carried out were the Best reaction for glycogen, the PAS reaction for polysaccharides, the methyl green reaction for DNA (desoxyribonucleic acid) and RNA (ribonucleic acid), the Feulgen reaction for DNA, the sulfhydryl reaction with Bennett's reagent and the reaction for disulfide groups with methylene blue and cobalt sulfide.

The *Best glycogen*[7] reaction is based on the staining of glycogen with carmine in its different concentrations. The cytoplasm of the cell is stained carmine red. The nucleus is unstained and may be counterstained with hematoxylin.

The result in normal tissue is a lack of staining material in the basal layers, and increased staining from the basal to the superficial epithelial layers. In carcinoma *in situ* we find no stain in the tissue. Cancer cells behave as non-differentiated basal cells, so that the amount of glycogen corresponds to the degree of differentiation of the cells.

The *PAS reaction* (McManus)[8] for polysaccharides can be chemically understood as an oxidation of glycol groups by periodic acid to produce aldehyde groups.

$$\mathrm{R-\underset{|}{\overset{OH}{CH}}-\underset{|}{\overset{OH}{CH}}-R' + HIO_4 \rightarrow}$$

$$\mathrm{R\overset{O}{\overset{\|}{C}}H + CHR'\overset{O}{\overset{\|}{}} + HIO_3 + H_2O}$$

The aldehydes which are formed are stained red by the leucofuchsin of the Schiff reagent. In normal squamous epithelial tissue the basal layer has little or no polysaccharides. Passing to the superficial epithelial layer the PAS reaction increases. In carcinoma *in situ* we find a negative reaction so that the amount of

polysaccharides corresponds to the degree of differentiation of the cells, the same as was found with the Best reaction. Not only polysaccharides but also mucoid substances, chitin, hyaluronic acid, heparin and chondroitin sulfuric acid are PAS positive. We find a strong reaction in endocervical glands because of the amount of mucoproteins in the cells. The cytoplasm is stained, the nucleus is unstained.

The *methylgreen pyronin*[9] reaction is used to stain the DNA of the nucleus green, the RNA of cytoplasm and nucleolus red. In carcinoma *in situ* there is an increase of both stains. Cancer cells show a moderate increase in stain intensity for DNA, a marked increase for the staining qualities for RNA of the cytoplasm.

The *Feulgen*[10] stain is based on the Schiff reaction for the aldehyde desoxyribose formed by acid hydrolysis from nucleoproteins. It stains red with leucofuchsin, and indicates the presence of DNA in the cell. In carcinoma *in situ* there is an increase in staining reaction as compared to normal cell nuclei. The nucleus is stained but not the cytoplasm. There is a distinct difference between the red stains in the Best, PAS and Feulgen reactions. The Best red is a carmine red, a light red color without clumps. The PAS color is much more intense, showing dark red clumping. The Feulgen red color is magenta red displaying a purple tendency with violet quality. It is possible to stain the nucleus of the cell with Feulgen and the cytoplasm with Best (the dual Feulgen stain) giving a demonstration of the different color qualities visible in the cells.

The *sulfhydryl (SH) groups*[11] present in animal tissue are bound to amino groups. The Bennett method is based on the reaction of mercaptans with SH groups. The synthesized 1-4 chloromercuriphenyl azo-2 naphthol or red sulfhydryl reagent (RSR) can be represented by the formula ClHgR′. The reaction with SH groups (R-SH) then proceeds as follows:

$$RS\,H + Cl\,HgR' \rightarrow RSHgR' + HCl$$

The reaction product is very slightly soluble and diffusible and it stains orange. In our tissues the reaction was most intense in the basal layers, weaker in the superficial layers and appeared slightly stronger in the cancerous tissue.

The method used for *disulfide (SS) groups*[6] was based on oxidation of cystine by peracetic acid. The oxidized tissue was then stained with 1/2000 M methylene blue at *p*H 2.6, or treated with 2 per cent cobalt nitrate followed by diluted ammonium sulfide to give blue or black staining respectively. In our experiments superficial cells were strongly stained as are some of the basal cell elements (cysteine groups being oxidized to cystine groups). The intermediate cell elements show very little stain. Cell formations of carcinoma *in situ* had a lower color intensity than the normal squamous cell formations. Pearl formations in cancerous tissue were strongly stained due to the large quantity of keratin.

Summary

A summary of the mentioned histochemical characteristics of carcinoma *in situ* tissue as compared to normal tissue shows the following:

Nonenzymatic reactions

Markedly increased	RNA (methylgreen-pyronin)
Moderately increased	DNA (Feulgen)
Slightly increased	SH groups (Bennett)
Markedly decreased	SS groups (Methylene blue and CoS)
Markedly decreased	Glycogen (Best reaction)
Markedly decreased	Polysaccharides (PAS of McManus)

In nonenzymatic reactions the cytoplasm is stained and not the nucleus, except in the Feulgen reaction where the nucleus is stained.

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THE histological diagnosis of carcinoma *in situ* of the cervix uteri depends upon the "subjective" appreciation of altered epithelial pattern, hypercellularity, loss of cell maturation and cellular atypia. Moreover, in the common basal cell type, it is necessary to demonstrate that cellular atypia due to complete failure of maturation is present throughout all layers up to the surface of the epithelium.[4] Accurate diagnosis is also dependent upon the amount and type of cervical tissue available for examination because adequate assurance must be provided that the basement membrane has not been breeched.

Unfortunately, the standard histological criteria set forth to distinguish advanced degrees of basal or reserve cell hyperactivity dysplasias and bizarre squamous metaplasia from carcinoma *in situ* are subject to errors of individual interpretation. The problem is of more than academic interest since pathologists and surgeons must have a mutual understanding of the condition, its histological criteria, and biological potential, if treatment is to be uniformly sound. To contribute to this end, we have studied certain histochemical properties of the cervical material submitted to the laboratory in an effort to distinguish between benign and malignant cells on the basis of objective findings. We have employed a variety of special stains for the purpose of identifying specific classes of substances in the various epithelia, but we have made no attempt to quantitate or visualize individual chemical compounds.

Glycogen in the epithelial cells of the female genital tract is associated with cell maturation, although minimal quantities may be demonstrated in "maturing" cells and in the central portions of certain well-differentiated squamous cell carcinomas.[2, 4] The demonstration of glycogen in the superficial cells in an area of presumed carcinoma *in situ* militates against this designation because, in simplest terms, the diagnosis is based upon the finding of complete failure of maturation throughout all layers. We have found that this objective approach to the cytochemical differentiation of mature, maturing and immature cells closely parallels the cytomorphological characteristics.

The periodic acid Schiff (PAS) stain proved to be the simplest and most reliable of the several technics employed to evaluate the degree of maturation of cervical epithelial cells. The deepest layers of the ectocervical epithelium (immature basal zone) do not contain PAS stainable mate-

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rial. Proceeding toward the surface, the cells normally contain a progressively increasing amount of substance which is PAS stainable. Also, the reserve cells of the endocervix contain no PAS staining material, but the mature epithelial cells of the glands take a heavy stain. Squamous cells in areas of metaplasia in the endocervix take the stain if the cells are mature. Thus, in cervical tissues showing basal cell hyperactivity, only the superficial mature and maturing cells are positively stained. In carcinoma *in situ* the entire thickness of the surface or gland epithelium is made up of immature basal or reserve cells without PAS stainable material. The stain also is remarkably effective in demonstrating the basement membrane beneath the cervical epithelium and thus may be helpful in excluding minute areas of stromal invasion. It should be pointed out that the Best carmine stain for glycogen reacts in the cervical tissues in a fashion almost identical to that noted for PAS, but the differentiation of the basement membrane is less well depicted.

Mature cervical epithelium does not stain with the basic fuchsin sulfurous acid reagent; this indicates an absence of free aldehyde in the tissue. The reactions noted above in mature cervical epithelium are dependent upon periodic acid oxidation of the Schiff's reagent. A positive reaction is considered to be specific for compounds possessing free 1, 2- glycol groups, unsubstituted or with an amino group, alkyl amino group, or carbonyl group substituted for one of the hydroxyls. Generally, only glycogen, mucopolysaccharides, mucoproteins, and mucins are present in sufficient quantity to give a visible color with Schiff's reagent.[1]

In an effort to distinguish the PAS reaction due to glycogen from that due to the mucinoid substance, the tissues are digested with diastase to convert the glycogen to substrates that wash out of the section. The stain due to glycogen will, therefore, not appear in the diastase-digested tissue.[3] When the cervical material was treated in this manner, only a part of the PAS stainable material was removed from the mature cells. Thus, mature epithelial cells of the cervix contain mucinoid substances as well as glycogen, while undifferentiated or immature cells are lacking in both.

The mucinoid substances present in the mature cells of cervical tissue were partially identified by noting the cytochemical reaction to alcian blue, acid fuchsin, and azure A. The positive reaction noted with each of these stains indicates that the mucinoid substances are principally mucopolysaccharides. To date we have not attempted to demonstrate individual chemical compounds in this class of substances or to identify other mucinoid materials that may be present in small quantities. Moreover, in the case of azure A, we recognize that this stain will not distinguish pentosenucleic acid (PNA) from the sulfuric acid esters unless PNA-ase digestion is employed.[5] These substances take a cyanophilic stain with azure A and are distributer rather diffusely in a bubbly fashion on the distal third of the cells, particularly in the mature columnar cells of the endocervical glands. This intracellular distribution appears to be different from that noted in the endometrial glands where one notices a course granular stippling of this material only at the peripheral margin of the cells.

Other histochemical technics are applicable for use in cervical tissues, such as dehydrogenase, alkaline phosphatase activity, phosphamidase, protein bound sulfhydryl and disulfide groups and others, but none of these produce distinctive staining patterns that are helpful in distinguishing hyperplastic from anaplastic growths. Certain ones of these are found in greater abundance in anaplastic lesions than in normal tissues of the same cell types, but

none of these reactions is peculiar to cancerous lesions or seems to connote any special cell function peculiar to these lesions. Moreover, with few exceptions, the chemical specificity and validity for localization of the intracellular level of most histochemical technics have come under critical scrutiny since the advent of microspectrophotometric methods which make it possible to study cell components and quantitate individual chemical compounds.

In summary, histochemical staining methods can often serve as important adjuncts to other technics in properly identifying cervical lesions that may defy classification with routine tissue preparations. Although there is no known histochemical reaction pattern peculiar to carcinoma, certain special staining methods will supply valuable information concerning the biological maturity of the cells under study. This is particularly important in the case of carcinoma *in situ* since the demonstration of immature cells throughout the entire thickness of the epithelium is one of the basic criteria necessary for the diagnosis. The periodic acid Schiff (PAS) method, which stains the glycogen and mucopolysaccharides of mature and maturing cells, proved to be the simplest and most reliable of the several technics employed to evaluate the degree of maturation of cervical epithelial cells. Carcinoma *in situ* lesions show a negative reaction to PAS throughout all layers similar to that found normally only in the immature basal zone of the ectocervix and the reserve cells of the endocervix. This approach to the cytochemical differentiation of maturation closely parallels, but lends objectivity to, the more subjective cytomorphological interpretations. In addition, the *in-situ* nature of the lesion is easily appreciated in most sections because the basement membrane is prominently stained by this technic. The Best carmine stain for glycogen reacts in the cervical tissues in a fashion almost identical to PAS, but the differentiation of the basement membrane is less well depicted. Other histochemical technics demonstrate an increase in certain enzyme activities in anaplastic lesions over that noted in normal tissues of the same cell types, but none of these produces distinctive staining patterns that are helpful in distinguishing hyperplastic from anaplastic growths.

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Discussion

Jean Berger, Basel, Switzerland: In my contribution elsewhere in this Symposium, I have dealt with the histochemistry of carcinoma *in situ* and invasive carcinoma. In this paper we have studied particularly the behavior of glycogen, the neutral and acid mucopolysaccharides, the phosphatase and also the sulfhydryl compounds.

We agree with Botella-Llusia that one may still demonstrate glycogen in immature invasive carcinoma. PAS-positive substances, i.e., neutral mucopolysaccharides, are above all still found in immature carcinomas. Phosphatases similar to DNA and RNA can be demonstrated when there is a marked growth tendency present.

We do not find significant differences between invasive carcinoma and carcinoma *in situ* by means of histochemical methods, with the exception that with aid of the demonstration of acid mucopolyproteides (alcian blue/PAS) the basal membrane can be demonstrated. It is interesting to note that around the invasive growths new fibers of acid mucopolyproteides are always formed.

Constantin Herovici, Villejuif, Seine, France: One always finds traces and often even marked deposits of glycogen and mucopolysaccharides in differentiated squamous carcinoma, particularly in the most differentiated zones. It is obvious that

one does not find these substances in carcinoma *in situ*, because it is neither differentiated nor mature.

It is not the place here to discuss the glycogen metabolism of invasive carcinomas. Therefore I will only report that I have found, in a series of 350 cervical carcinomas, using the carmine stain of Best and the PAS reaction with and without digestion, that the more mature carcinomas are more rich in glycogen than the immature carcinomas. The presence of glycogen in the latter is for absolute exception. This also seems to be the opinion of Foraker, Nesbitt and Stein; however, it is not in accordance with Botella-Llusia.

It seems important to me to come to an agreement in regard to this point, for the absolute absence of glycogen and glucopolysaccharides in carcinomas *in situ* reflects its extreme immaturity. Probably this is its sole, precise histochemical characteristic, and thus it could be of use in the histological routine.

Closing Remarks

José Botella-Llusia: The opinions of Berger and Herovici seem contradictory. While Berger thinks that there is glycogen in the immature carcinoma, Herovici believes that glycogen is present in the carcinomas with a higher grade of maturation. Our search shows that carcinoma *in situ* does not show glycogen, a statement in which, with rare exception, there is general agreement. We find glycogen in invasive carcinomas with both immature and moderately mature, but not in the highly mature forms. Our opinion is that glycogen is not characteristic either of the immature carcinoma or of the highly mature carcinoma, but is present in maximum amount in the intermediate type of maturation. Similar phenomena in the normal ectocervical and vaginal epithelium take place: the basal layer does not have glycogen, the intermediate layer has a maximum amount, while the horny layer has a PAS-positive substance, which cannot be identified as glycogen since it is resistant to the amylase, as we have demonstrated previously (Botella-Llusia, J. and F. Nogales: Acta Cytol. **2:** 263, 1958).

Carcinoma *in situ* could be, thus, representative of the basal layer, as the intermediate zone could be the "semimature" carcinoma, as the superficial horny layer could be the representative of the pearl formations of the mature squamous carcinomas.

B. Cornelis Hopman: Although some glycogen may be present in undifferentiated invasive carcinoma, I found the amount markedly decreased as compared to the normal differentiated epithelium both with the Best carmine and PAS reaction (Hopman, B. C.: Am. J. Obst. & Gyn. **79:** 346, 1960).

Phosphatases were found to give a quantitatively increased reaction in malignant tissue; however, qualitatively there was no significant difference between cancer cells and normal basal cells.

Robert E. L. Nesbitt, Jr.: We are convinced that the diagnosis of carcinoma *in situ* depends upon the demonstration of cellular atypia due to complete failure of maturation of cells throughout all layers of the cervix from the intact basement membrane up to the surface of the epithelium. Errors of individual interpretation occur when this diagnosis is based solely upon the study of cervical material prepared in the routine fashion utilizing only standard histological technics. The failure to demonstrate PAS stainable material (glycogen and glucopolysaccharides) throughout all layers of the cervical epithelium lends a high degree of objectivity to these interpretations. A definitely positive PAS reaction which indicates the presence of "mature" or "maturing" cells is not compatible with the diagnosis of carcinoma *in situ*. The latter lesion is always an immature carcinoma and, in our experience, characteristically does not take the stain. The use of this technic as a means of studying atypical lesions of the cervix has become a valuable routine that supplements conventional methods of study in our department.

A Study of Basement Membranes of Normal Epithelium, Carcinoma *In Situ* and Invasive Carcinoma of Uterine Cervix Utilizing Electron Microscopy and Histochemical Methods

CHARLES TEMPLE ASHWORTH, VERNIE ALBERT STEMBRIDGE, FRANCIS JOSEF LUIBEL

THE differentiation between early invasive squamous cell carcinoma and carcinoma *in situ* of the cervix is frequently difficult, and in some instances cannot be accomplished with certainty, using customary histologic technics. Since metastatic spread is always a possibility if infiltration of the stroma can be demonstrated, the recognition of the earliest stages of invasion is of considerable practical significance, and may have a bearing upon the type of therapy to be employed in cases of cervical carcinoma.

The status of a basement membrane in cervical mucosa, until recently, has been uncertain, since some observers[1] maintained that it existed, and others[2] refuted its presence. Dougherty and Low[3] however, have recently clearly demonstrated a basement membrane in normal cervical epithelium with electron microscopy, and this observation has been confirmed and extended.[4] The determination of the invasive status of cervical carcinoma usually is considered to depend upon whether or not the neoplastic cells are confined by the underlying stroma and the basement membrane,[5] although the demonstration and even the presence of the latter is usually based on presumptive evidence.

Since the basement membrane is readily depicted by electron microscopy, and since a condensed membrane, comparable in location to the basement membrane of electron microscopy, can be demonstrated by several histochemical procedures, these methods were utilized in a comparative study of normal and carcinomatous cervical epithelium. An effort was made, on the basis of these studies, to define the morphological aspects of the ultrastructural level of invasion of cervical carcinoma, and to determine the part played in this process by the basement membrane and the connective tissue stroma.

Methods

Tissue for electron microscopy was obtained immediately, at the time of biopsy of the cervix or hysterectomy, from four normal cervices, and three each of carcinoma *in situ* and invasive carcinoma. Blocks of approximately 1 cu. mm. were fixed in a chrome-osmium solution[6] for two hours, passed through alcohols, and embedded in methacrylate.[7] Ultra-thin sections about 1/40 μ thick were prepared with a Porter-Blum microtome, using a diamond knife.[8] These preparations were studied with an RCA EML or RCA EMU-3 microscope.

Microscopic sections were prepared from the same specimen, utilizing paraffin embedded tissue, obtained from the same areas as that used for electron microscopy. In addition, histological sections were prepared from still another group, including four normal cervices, five cases of carcinoma *in situ*, and six cases of invasive cervical squamous cell carcinoma, of which three were in an early stage of invasion. The histo-

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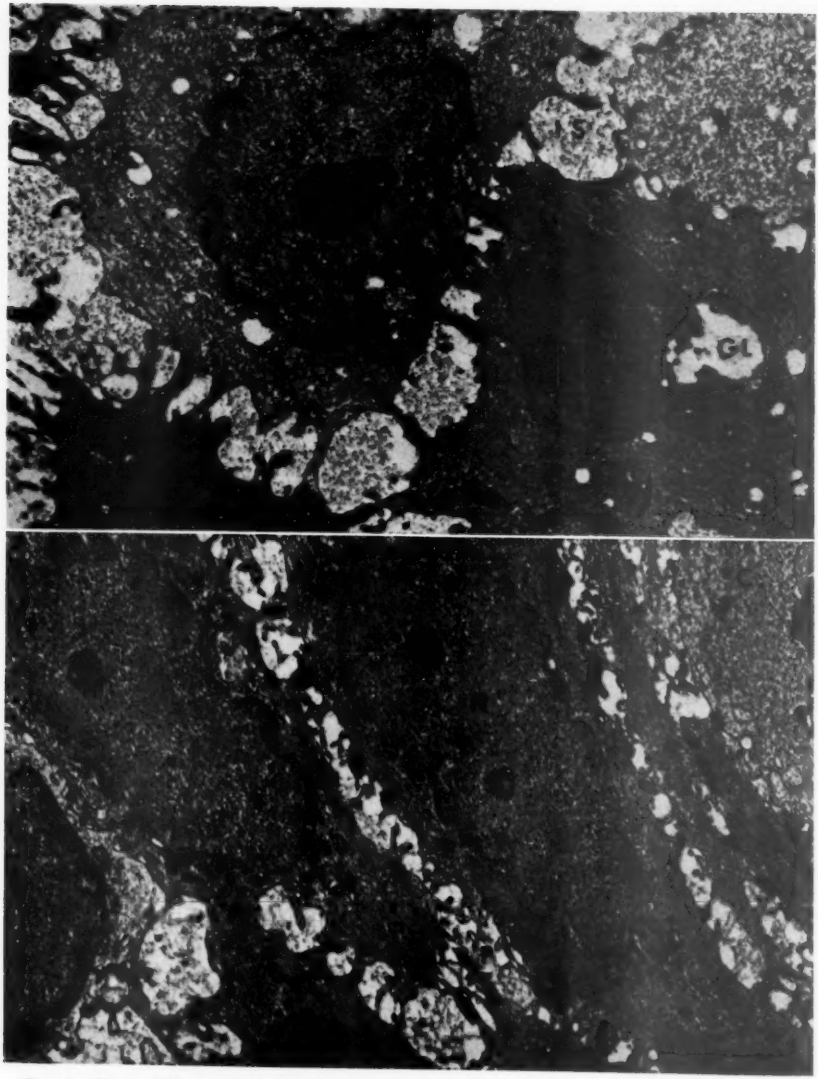

FIG. 1 (Top). Electron micrograph of normal cervical epithelium. Precornified cell zone. Wide intercellular spaces (IS) are present, and intercellular bridges can be seen. A glycogen droplet (GL) is located in the cytoplasm near the nucleus. The nuclear membrane has a moderately irregular contour. One-micron markers are located in the lower left hand corner of the electron micrographs (x5400). FIG. 2. Electron micrograph of carcinoma *in situ*. Cells in midzone of squamous epithelial layer. In addition to intercellular bridges (IB) there are many fine villous projections (P) at the cell membrane. The nucleus (N) is enlarged, and the nuclear membrane is somewhat folded. A portion of a degenerating cell may be seen (x5400).

logical sections were subjected to the following procedures: hematoxylin-eosin stain; periodic acid-Schiff[9] (PAS); PAS after incubation with diastase;[10] PAS with alcian blue[11] (PAS-AB); colloidal iron with PAS, and control;[12] azure B stain; and azure B after incubation with ribonuclease, for ribonucleoprotein;[13] and a histochemical procedure for sulphydryl.[14]

Results

Those observations pertaining to the electron microscopic findings of the cell structure and intercellular connections of normal cervical epithelium, carcinoma *in situ*, and invasive carcinoma have been incorporated in previous reports.[4, 15] In summary, these studies revealed in carcinoma *in situ* and invasive carcinoma cells, as contrasted with the normal epithelium, a condensation of nuclear granules (probably DNA protein) just inside the nuclear membrane and around enlarged nucleoli; prominent clefts and infoldings of the nuclear membrane; scarcity or absence of glycogen droplets within the cytoplasm; enlargement and vacuolization of mitochondria; increased density and clumping of RNA protein granules; presence of villous-like formations at the cytoplasmic membrane; and decreased number and imperfections of the intercellular bridges (Fig. 1-3). The present report will be concerned with the basement membrane area, and the junction zone between the epithelial cells and adjacent stroma, as they are related to the status of invasion.

Electron microscopy clearly reveals a continuous basement membrane between the basal cells of normal cervical epithelium and underlying stroma (Fig. 4). This membrane is estimated to be about 300 Å thick. It is a homogeneous structure, but on the stromal surface, fine granules and fibrils are noted, which suggest a relationship to collagen fibrils. A space of about 200 Å width is present between the basal cell cytoplasmic membrane and the basement membrane. In normal epithelium, the basement membrane is markedly folded and convoluted. This is due to the presence of numerous, fine, foot-like projections of the basal cells. This arrangement at the basal surface of the epithelial cells is suggestive of an anchoring effect.

Although the electron-dense structure identified in electron microscopy as basement membrane is too thin to be visible in light microscopy, a comparable but thicker lamina is demonstrable by any of the histochemical procedures designed to identify acidic polysaccharides. Thus, the PAS, PAS-AB, and colloidal iron procedures reveal a distinct and continuous lamina in normal cervical squamous epithelium (Fig. 5). However, in a few areas it is converted into a fine network or appears frayed, due to tangential cutting and therefore an intact membrane cannot always be traced out. Azure B stains, controlled with parallel preparations previously incubated with ribonuclease, reveal a small zone of homogeneous, compact cytoplasm (RNA protein) between the nucleus and the basement membrane. No azure B-stainable material is noted beyond the basement membrane.

In carcinoma *in situ*, electron microscopy shows a continuous intact basement membrane, the structure and thickness of which are similar to normal (Fig. 6). However, there is a notable straightness of the basement membrane, reflecting an absence of the foot-like projections of the basal cells. PAS, PAS-AB, and colloidal iron procedures also demonstrate a stainable membrane, as in normal squamous epithelium (Fig. 7). In those cases where extensive mucous gland encroachment has occurred in carcinoma *in situ*, these histochemical procedures demonstrate clearly the persistent and intact basement membrane (Fig. 8). The PAS-AB stain in such cases also usually demonstrates small remnants of mucus within the almost solid cell masses, as alcian-blue-positive, PAS-negative material. In some of the more active appearing carcinomas *in situ*, PAS-, AB-, and colloidal iron-positive material

may be seen, incorporated within the epithelial layer (Fig. 9). Close study shows these to be strands of connective tissue, with or without capillaries, which have been incorporated within the epithelial layer. In some instances, continuity of these incorporated structures with adjacent stroma can be demonstrated.

In invasive carcinoma, several distinctive alterations of basement membrane are observed. Electron microscopy shows most of the infiltrating cell groups to be devoid of a basement membrane (Fig. 10). The PAS, AB, and colloidal iron stains confirm this observation in histologic sections. The junction zone between most neoplastic cell groups and stroma, in these preparations, is less sharply demarcated than in normal epithelium and carcinoma *in situ*, and a membrane cannot be seen (Fig. 11). Where the basement membrane is missing, there is usually a slight separation of the tumor cell group from the surrounding stroma. No doubt, this is an artifact, but it may be due to decreased adhesiveness of cells to stroma, in the absence of basement membrane. Although the neoplastic cell groups of invading carcinoma usually are not surrounded by basement membranes, there are some exceptions. In these, electron microscopy (Fig. 12), and histochemical preparations reveal varying amounts of investing basement membrane.

Frequently, around invading carcinoma cell nests, the PAS stain reveals a thick, condensed zone, somewhat resembling basement membrane. Careful study of this PAS positive layer, and evaluation of the alcian blue and colloidal iron stains, indicate that this is usually a compressed layer of stromal elements, rather than true basement membrane, however.

Within the infiltrating carcinoma cell masses, the PAS and other stains for mucopolysaccharides, reveal incorporated strands of connective tissue and of capillaries (Fig. 13). These are more numerous, and more deeply situated, than similarly incorporated stromal elements in the *in situ* carcinomas. Occasionally also, the PAS stain reveals a fine, linear, fiber-like material between individual cells, which is unaffected by diastase.

Another aspect of the carcinoma cells, at the zone of junction with stroma, which is revealed by electron microscopy, is the occasional strand-like protrusion of neoplastic cell cytoplasm into the adjacent stromal spaces (Fig. 14). These projections may sometimes be traced to the cell body from which they arose, in the carcinoma cell nest. The cytoplasmic projections arise at various points along the border of the tumor cell group, where basement membrane is absent. Lipid droplets are commonly noted in these strands of cytoplasm.

Azure B stains were utilized in an attempt to visualize the light microscopic aspects of the cytoplasmic protrusions seen with electron microscopy. When controlled with ribonuclease-incubated sections, azure B stainable material may be identified as ribonucleoprotein. Since the cytoplasm of cervical carcinoma cells has densely arranged, clumped RNA protein granules,[15] the azure B stains are especially good to demonstrate the location and distribution of cytoplasm of these cells. At the margins of invading carcinoma cell groups, it is sometimes possible to find clumps of azure B-stainable material among adjacent stromal fibers (Fig. 15). These are believed to represent extensions of cytoplasm, comparable to that visualized with electron microscopy. In contrast to cytoplasm of carcinoma cells, the stromal cells contain a small zone of homogeneous, compact azure B-stainable cytoplasm, confined to the immediate perinuclear area.

In histologic sections of normal cervical epithelium, carcinoma *in situ*, and invasive carcinoma, it frequently is possible to evaluate the effects of inflammation upon basement membrane. In hematoxylin-eosin stained sections, the inflammatory cells sometimes obscure the junction area between neoplastic cells and stroma. However, even in the presence of abundant inflammatory exudate, the PAS, AB, and

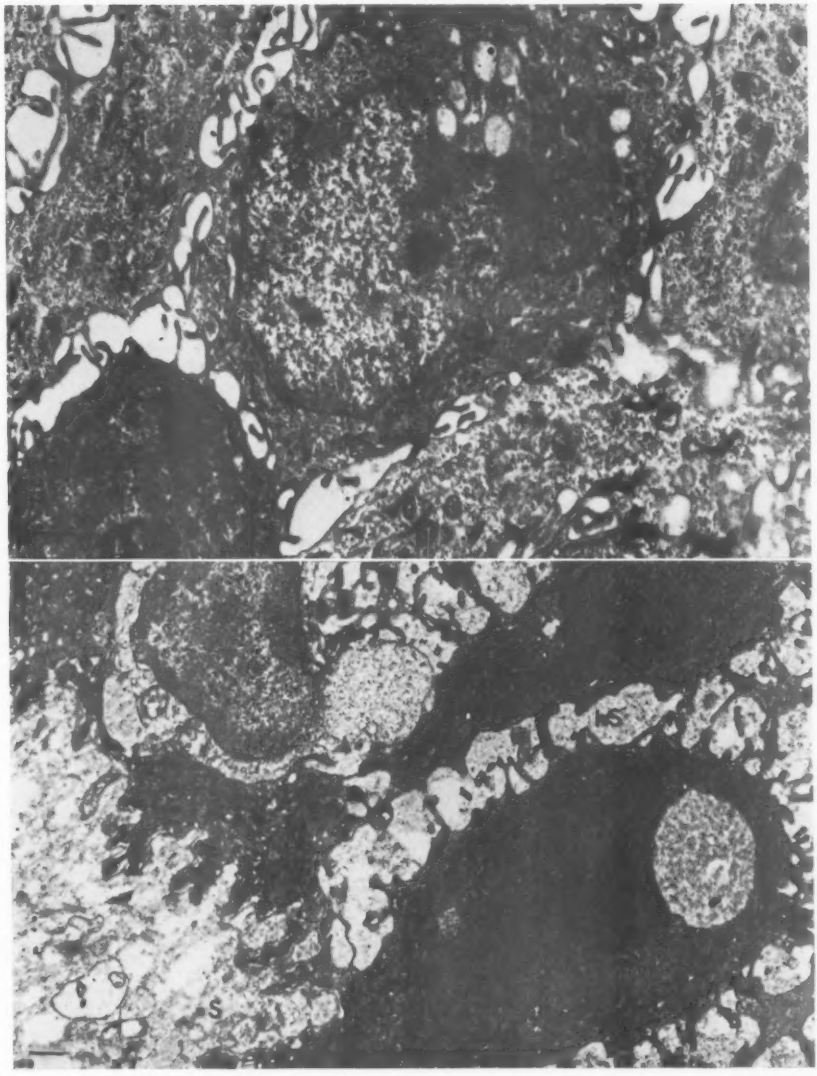

FIG. 3 (Top) Electron micrograph of portions of cells from an invasive squamous cell carcinoma of the cervix. The large nucleus contains condensed areas of chromatin granules. The cell membrane contains several intercellular bridges and numerous fine villous projections (P) (x5400).
FIG. 4. Electron micrograph of basal cell layer of normal cervical squamous epithelium. The basement membrane is markedly folded, due to the projecting foot processes (F) of the basal cells. Wide intercellular spaces (IS) are noted at this level of the epithelium. A small zone of stroma (S) is present. One cell contains a small droplet of glycogen (x5400).

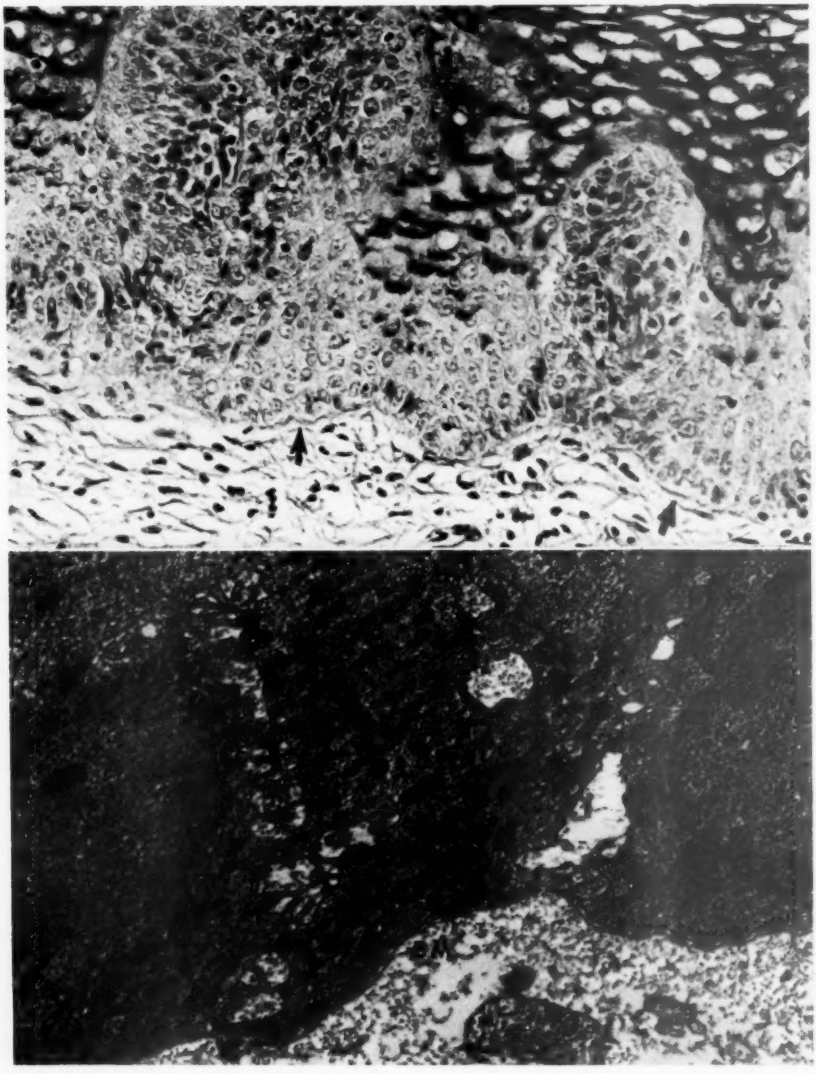

FIG. 5 (Top). Colloidal iron -PAS stain of normal cervical squamous epithelium. The basement membrane is visible at arrows as a dark, fine line along the basal cell layer (x300). FIG. 6. Electron micrograph of carcinoma *in situ* of cervix. The basement membrane (BM) is apparent as a rather straight line. A narrow lucent zone is also visible between the cell membrane and the basement membrane. Dark mitochondria (M) are visible in the basal layer of epithelial cells (x5400).

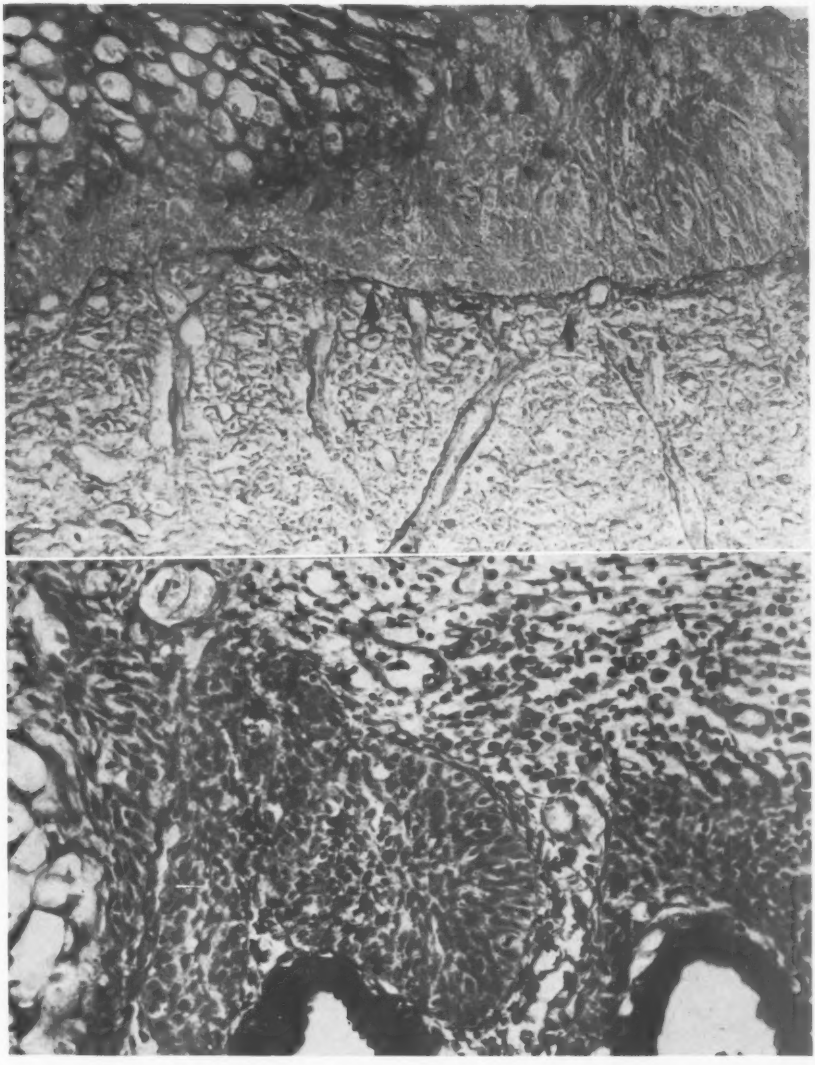

FIG. 7 (Top). PAS–hematoxylin stain of carcinoma *in situ*. Photomicrograph shows normal squamous epithelium on the left side and carcinoma *in situ* on right side with the dark line (arrows) along the base of the epithelium indicating the presence of a continuous basement membrane (x300). FIG. 8. Colloidal iron-PAS stain of carcinoma *in situ*. The site of neoplastic cells which have extended into subajacent mucous gland is indicated by an arrow. A dark line surrounding these cell groups indicates the presence of an intact basement membrane (x300).

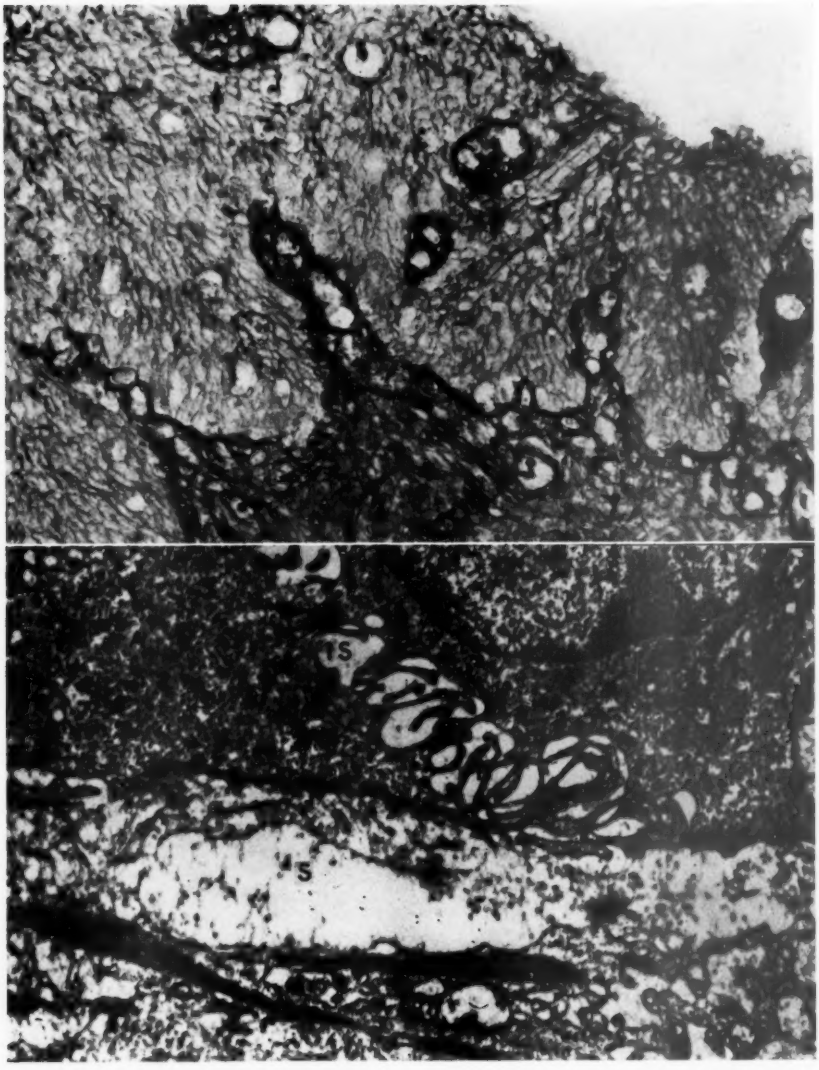

FIG. 9 (Top) PAS stain of carcinoma *in situ*. The arrows indicate stroma and capillaries which have been incorporated in the layer of epithelium, which however is still limited by a stainable basement membrane (x300). FIG. 10. Electron micrograph of invasive squamous cell carcinoma. Portions of two cells (A and B) are represented. A wide intercellular space (IS) is located between the cells, and numerous cytoplasmic projections (P) are found. At the junction (J) of stroma (S) and neoplastic cells, no recognizable basement membrane is apparent (x8,000).

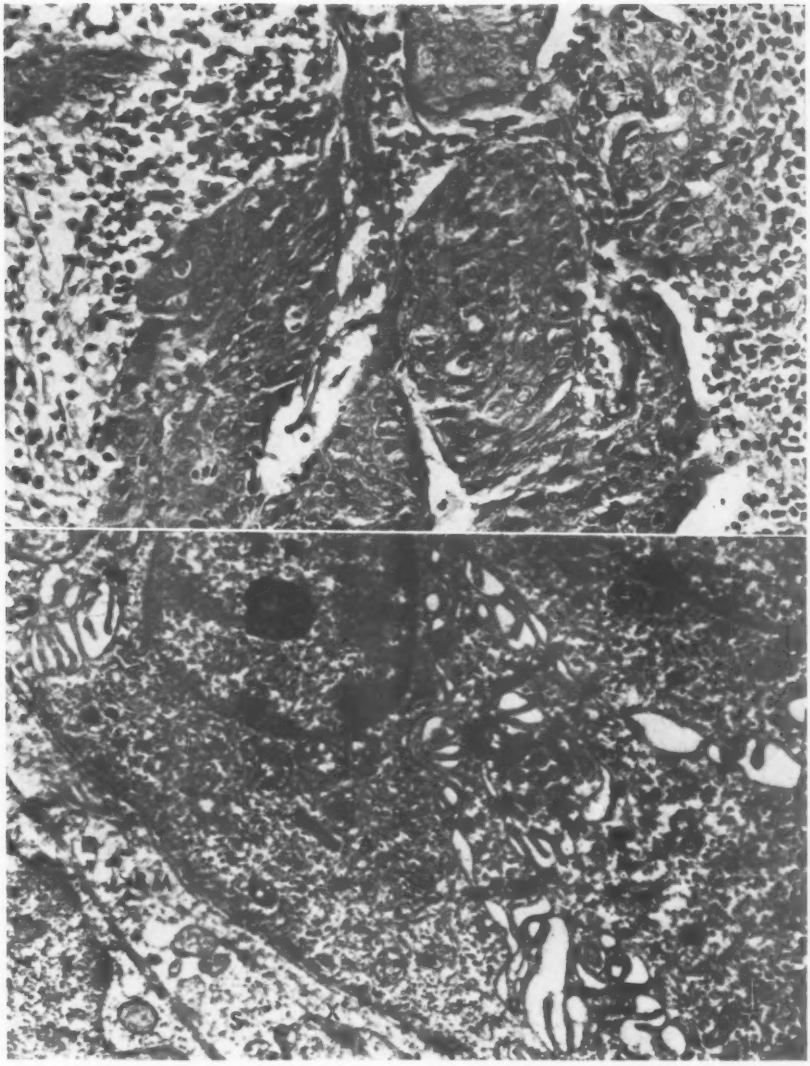

FIG. 11 (Top). Colloidal iron PAS stain of invasive squamous cell carcinoma. The junction zone between stroma and neoplastic cells is less sharply demarcated than normal, and no basement membrane is apparent (x300). FIG. 12. Electron micrograph of invasive carcinoma. A basement membrane (BM), showing slight, focal imperfections (X) is present at the junction between carcinoma cells (A) and stroma (S) (x8,000).

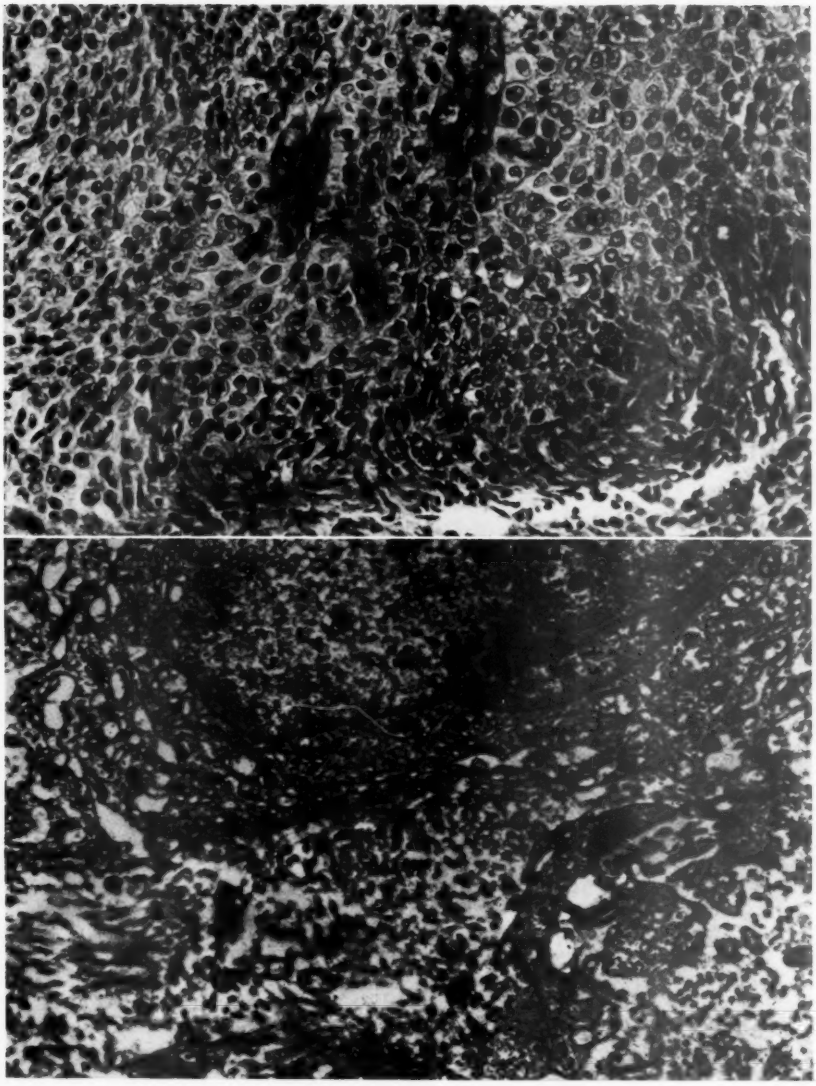

FIG. 13. (Top). Colloidal iron PAS stain of invasive squamous cell carcinoma. Stroma and capillaries are found (arrows) incorporated within the neoplastic cell groups (x300). FIG. 14. Electron micrograph showing junction between neoplastic cells of invasive carcinoma and stroma (S). There is no visible basement membrane. There is a long strand of cytoplasm extending into the connective tissue spaces. Within this cytoplasmic extension, lipid droplets (L) are apparent (x8,000).

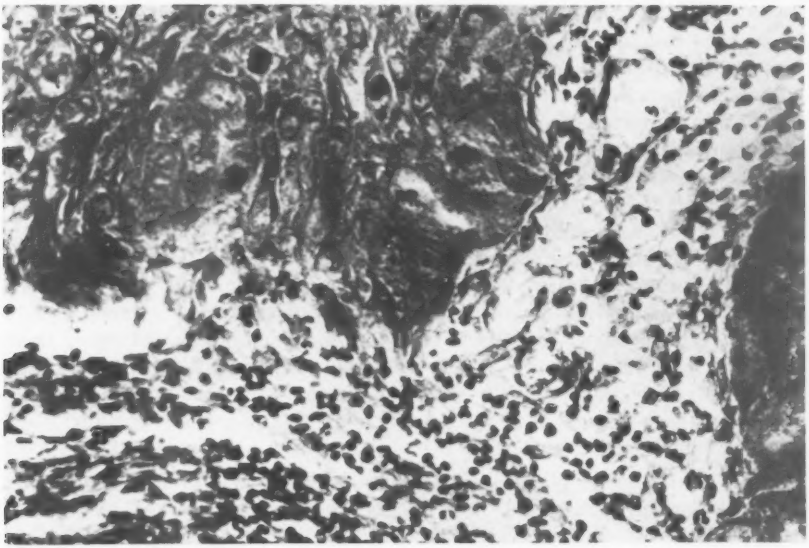

FIG. 15. Azure B stain of invasive carcinoma. At arrow are RNA protein-containing, cytoplasmic protrusions of neoplastic cells into the stroma (x400).

colloidal iron stains show the membrane to persist, and to remain intact.

Discussion

Since the thickness of the basement membrane visualized with electron microscopy (300 Å) is below the level of light microscopy, it is necessary to inquire about the identity of, or relationship between the membrane depicted by histochemical means, and that seen with the electron miscroscope. The identity of structure and similar dimensions of the basement membranes of glomeruli and other capillaries, and that seen in cervical epithelium,[1, 4, 16, 17] indicate that the membrane of electron microscopy is actually a true, structural membrane. The greater thickness of the membranous layer, which is visualized by histochemical localization and light microscopy, may be explained, in part at least, by the assumption of an electron-lucent, cement material located around the electron-dense basement membrane. This would add considerably to the total thickness of histochemically demonstrable membrane. This is also true in the case of glomerular capillary basement membrane.[16] The presence of a 200 Å wide lucent zone, between the electron-dense membrane and the base of the cervical epithelial cells, supports this concept. It is also probable that the localization of stain on the membrane is such that it would, in itself, thicken the visible zone. These considerations, and the demonstration of the parallel occurrence of the histochemically and electron microscopically demonstrable membranes, in normal cervical epithelium and cervical carcinomas, provide a sound basis for assuming the two structures to be essentially synonymous, with the reservations as indicated.

Although it has been generally believed that the basement membrane serves as a deterrent to stromal penetration by neoplastic cells, actually there has been little real proof of this. The observations reported herein, on the other hand, do emphasize a correlation between invasive properties and the absence of basement membrane, as well as a correlation of noninvasion with the presence of basement

membrane. Cytoplasmic penetration of the stroma, probably the first step in invasion, was demonstrated to occur only at those sites where the basement membrane was defective. Even assuming that loss of basement membrane is a necessary prelude to invasion, the reason for the disappearance of the membrane is, most assuredly, not apparent. It might be assumed that the malignant neoplastic cells in some way mechanically disrupt and destroy the membrane, or that they produce a metabolite capable of depolymerizing or otherwise effecting a loss of continuity of the membrane. There is even the possibility that the connective tissue stroma is altered in such a way that it fails in its perpetual reconstruction of the basement membrane. Since carcinoma *in situ* is a recognizable entity in the cervix, and may persist as such for many years[18] without undergoing invasion, speculation upon the mechanisms which might be involved in leading to the invasive state, are not perhaps without biological significance at this time.

The observation that some deeply infiltrating carcinoma cell groups may be invested by partial or complete basement membrane, requires some consideration. Since this occurs in some instances, the demonstration of intact basement membrane, either histochemically or with electron microscopy, cannot be unreservedly interpreted to indicate that a given nest of neoplastic cells is of non-invasive status. Evidently, as tumor cells develop a new locus in the invaded stroma, basement membrane material is produced, either from the neoplastic cells, or more likely, from the connective tissue itself. Whenever invading cell nests are so invested by basement membrane, it seems probable that the invasive process is at a relative standstill. It would appear worthwhile to evaluate this process of basement membrane investment in invasive carcinomas against their biological behavior, prognosis, and the effects of irradiation therapy, in individual cases.

It might very well be asked, what actually constitutes invasion in carcinoma of the cervix? May it be assessed as a phenomenon marked by an overt single behavior characteristic, or is it a gradual process without sharp limitations? The answer appears to be a matter of definition. The overtly invasive[19] carcinoma of the cervix is readily recognized by gross and histologic study, as one where the invading neoplastic cell groups have extended deeply into the stroma, and are in no way connected with surface epithelium or cervical glands. Recently, a microinvasive[20] stage of carcinoma has been discussed. This concept is based upon the interpretation of histological sections in which it is not possible to differentiate with certainty between a poorly demonstrable junction of carcinoma cells with stroma, and true penetration of the stroma, beyond the confines of the basement membrane. The correlated electron microscopic and histochemical study reported here, provide tangible evidence for the fine microscopic, and ultrastructural stage of invasion. This stage is indicated by the disappearance of basement membrane, and by the penetration of strands of cytoplasm into adjacent connective tissue spaces.

In cervical carcinoma *in situ,* as well as full invasive carcinoma, fine strands of connective tissue and capillaries incorporated within the epithelial layer can frequently be demonstrated by histochemical means. It should be determined if possible, whether this represents true stromal invasion, since it might be a criterion of practical applicability for the differentiation of invasive from noninvasive carcinoma. In normal cervical squamous epithelium, it is possible to find extensions of connective tissue and blood vessels from the underlying stroma. These are quite analogous to the papillary bodies of epidermis. Many of the strands of stromal tissue and capillaries in invasive carcinomas, and in carcinoma *in situ,* may be found to be continuous with adjacent stroma. It is assumed that this could be done for virtually all such isolated strands upon study of serial sections.

Thus, these extensions apparently represent only an exaggeration of the vascularity of cervical epithelium, and are not necessarily indicative of true invasion. It is entirely conceivable that the stroma and capillaries may be pulled up into the neoplastic cell mass without the basement membrane ever having been disrupted, and without penetration of neoplastic cells into stromal interstices.

It is felt that these observations upon the ultrastructural stage of invasion of cervical carcinoma are primarily of interest as additional perspectives in the behavior of malignant neoplasms. However, a practical question arises from the consideration of these observations, *viz.*, whether the application of histochemical methods for basement membranes, with or without electron microscopic study should be a part of the routine pathologic study of carcinoma *in situ* or questionably invasive carcinomas of the cervix. The present impracticality of any significant sampling of the lesion for electron microscopy, indicates that this method of study would not be useful at this time. The demonstration of basement membrane by histochemical procedures, and the study of cytoplasmic extension into stroma with azure B or other comparable RNA protein stains, however, could be applied very readily to appropriate sections. As an additional means of study in such cases, it is believed that these procedures should be utilized more frequently. However, in their interpretation, certain limitations should be appreciated. For one thing, due to tangential cuttings, it is not always possible to trace perfect basement membrane continuity, even in normal stratified squamous epithelium. Also, some nests of invasive carcinoma, perhaps relatively inactive as far as invasive behavior is concerned, develop a new basement membrane. Furthermore, the question must be entertained, whether the recognition of such very early invasion is of practical value in terms of therapy. It has been observed[21] that lymph node metastases are present only exceedingly rarely even in frank, but minute invasive cervical carcinoma. For the sake of lesser morbidity from therapy, it might be desirable that the stages of early invasion discussed here should be treated as carcinoma *in situ*. Still, even though this might be true, the recognition of this stage of invasion is of practical importance, since it indicates that the long latent period of noninvasion has passed in that particular case, and that overt invasive carcinoma is not far removed, in all likelihood.

Summary

A comparative study of normal cervical epithelium, carcinoma *in situ*, and invasive cervical carcinoma was carried out, using electron microscopy and histochemical procedures for basement membrane and cytoplasmic RNA protein. Special attention was given to the study of basement membrane and the zone of junction between neoplastic cells and stroma.

It was found with electron microscopy, that a convoluted basement membrane of about 300 Å thickness was present in normal epithelium, and a straight but otherwise similar membrane occurred in *in situ* carcinoma. In invasive carcinoma, basement membrane was absent around at least some of the invading cell groups. However, carcinoma cell groups were also found that were partially or completely invested by basement membrane which evidently was newly formed.

Histochemical studies—PAS, alcian blue, and colloidal iron stains, confirmed the presence or absence of basement membrane as seen electron microscopically. Although the true basement membrane of electron microscopy is too thin for light microscopic visualization, reasons for its demonstrability with the histochemical procedures are discussed.

Observations with electron microscopy, and with azure B stains revealed areas of penetration of the stroma by cytoplasmic strands from the neoplastic cells. This occurred in the absence of basement mem-

brane. The earliest phase of invasion is believed to be represented by this process. The significance of this concept of early invasion is discussed as it is related to the biologic characteristics of cervical carcinoma, and as it may be related to diagnosis and therapy.

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We wish to acknowledge gratefully the technical assistance of Miss Nancy Arnold in the histochemical studies, and Mr. Emil Sanders in the electron microscopic preparations.

Discussion

Gunter F. Bahr, Stockholm, Sweden: This is certainly an important contribution to our understanding of the function of the basement membrane in general and particularly with regard to its role during the invasion of cancer cells. The special properties of this extracellular structure as a boundary between epithelium and underlying connective tissue, or separating different cell types (kidney), have become the focus of interest in an increasing number of studies.

The apparent discrepancy of membrane thickness in the electron micrograph and the light microscope image is discussed by the authors. To this it might be added that the electron micrograph has a much higher geometrical resolution (200Å—300Å section thickness) while the light microscope summarizes the foldings and waves of between 300 and 200 of these thin sections in a, *e.g.*, 6μ thick section. With high power the depth of focus is about 0.1μ *viz.* corresponding to at least 4-5 ultra-thin sections.

It is also justified to assume that the black and white representation of the electron scattering properties of the object, *viz.*, the micrograph may contain material which cannot be demonstrated electron-optically. There is no doubt, however, that the 300Å thick basement membrane in the electron micrograph is part of the histochemically demonstrable analog.

Since the loss of certain structural detail in epithelial cells can be interpreted as steps of dedifferentiation, it would be interesting to know if the authors can correlate a certain cell type to places where imperfections or nonexistence of the basement membrane exists. Studies of biopsies from carcinoma *in situ* indicate that the growth rate and the exfoliation rate of atypical epithelial cells may vary appreciably. This in turn will cause varying degrees of local nutritional stress and disorders in the supporting tissue, thereby influencing the properties of the filtering barrier, the basement membrane.

Carlo Sirtori, Milano, Italy: I wish to express my congratulations to Ashworth, Stembridge and Luibel for their very important histological, histochemical and electron microscopic investigations.

I have observed some cases of metastases of squamous cell carcinoma to lymph glands in which the nests of metastatic cells were surrounded by a basal membrane (Fig. 1, 2).

The metastases were formed by several layers of cells, as in a normal epithelium and the basal membrane was situated between the basal cell layer and the underlying connective stroma. I am very glad that my observation has now been confirmed by the above authors' electron microscopic studies. I believe that in such cases the metastases of the tumor differentiated and its stroma became organoid. One could assume that under certain conditions epithelial tumor cells and stromal connective tissue may interact and produce a basal membrane, in the same way as they form tonofibrils and desmosomes.

For a better comprehension of the persistence of the basal membrane in invasive cancers, we have to remember that in normal tissues the basal membrane is considered the product of an interaction between the overlying epithelium and the underlying connective tissue. When cancer cells are so

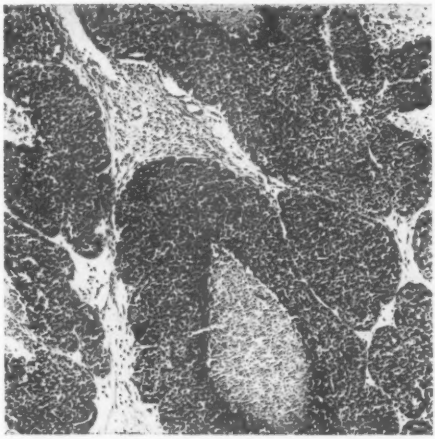

FIG. 1. Lymph gland metastasis of squamous cell carcinoma (Hemat.-eosin stain). (Sirtori)

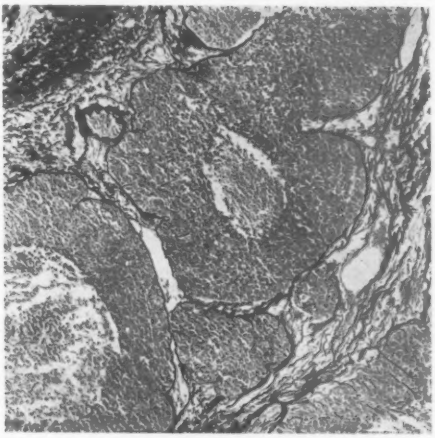

FIG. 2. The same case as Figure 1. (Silver stain shows the basal membrane between the tumor cells and connective stromal tissue.) (Sirtori)

altered that they are unable to collaborate in the formation of the basal membrane, the tumor becomes infiltrating. When the connective tissue is damaged and unable to participate in the formation of the basal membrane the cancer becomes infiltrating. It is necessary to consider both these situations in order to explain the biology of carcinoma *in situ*.

Hans-Klaus Zinser, Cologne, Germany: We have been engaged in studies on the fine structure of the vascular system of the cervical surface in order to learn about the peculiarities of the capillary system in normal and abnormal epithelia. We have been inspired to institute these kinds of studies by the numerous vascular pictures of the uterine cervix as revealed by the colposcope. It is known that one may recognize certain anomalies of the capillary system with the colposcope which have to be correlated to carcinomatous epithelial changes. In order to put these observations on an objective basis we have prepared the vascular system of the cervix, according to the method of Spanner, on 120 surgical specimens and have examined them under the stereo microscope. One histological section was prepared from each specimen so that we could compare the colposcopical appearance with the histological findings. The blood vessels were demonstrated up to their terminal branches on the inspected preparations. Figure 1 demonstrates the behavior of the capillary system of a normal squamous epithelial lining. These pictures are different from the characteristic capillary formations seen in the areas from the uterine cervix covered with glandular epithelium. During the process of epidermization a more or less pronounced vascularization of the ectocervical periphery takes place.

The various shapes and interrelationships of the capillary formations characterize the process of the ascending epithelialization. Atypical epithelial proliferations are distinguished by characteristic peculiarities of their terminal capillary system, as demonstrated in Figure 2. In this case we deal histologically with a small carcinoma, on the marginal regions of which a carcinoma *in situ* (Ober flächencarcinom) can be recognized.

In the injected specimen the dense, spongy vascular tree of the neoplasm is demonstrated (at the right of Fig. 2). Adjacent to this an irregular vascular network is found, extending to the epithelial surface (in the center of Fig. 2). As shown by the histological section, these peculiarities are correlated to a beginning infiltrative stage. In the left portion of the figure this irregular vascular network ends and changes into tightly standing, needle-like capillaries, this being a picture which corresponds to an increased atypical epithelium.

Our investigations speak in favor of the assumption that atypical epithelial changes go together with anomalies in the terminal vascular apparatus, which may have, as yet, an unknown significance in the scarring of the squamous epithelium. In a work soon to be completed we will discuss more thoroughly this interrelationship between the capillary system and the development of carcinoma.

Closing Remarks

Charles T. Ashworth, Vernie A. Stembridge and Francis J. Luibel: The suggestion of Bahr that local neoplastic cell alterations may be related to imperfections of the basement membrane is intriguing. We have not as yet been able to see any structural differences in the tumor cells at areas where the membrane is defective, as compared with cells associated with a well-preserved membrane. Additional observations along this line are indicated, however.

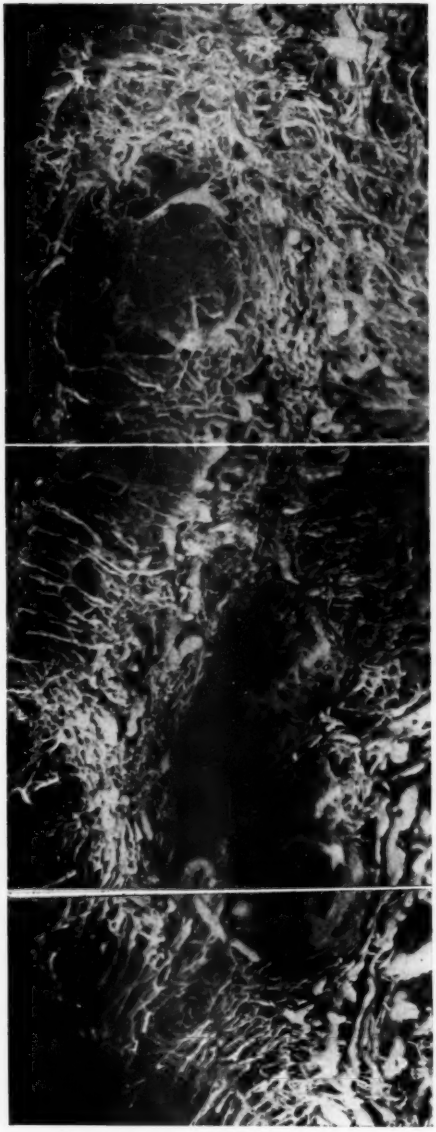

FIGURE 1 (Zinser)

Sirtori's comments on his observations of basement membrane around groups of infiltrating carcinoma of the cervix are especially helpful. We regret we were not previously aware of his demonstration of this phenomenon, but it strengthens the viewpoint that basement membrane may be produced by deeply infiltrating cell nests. In the electron microscopic study of other human malignant neoplasms, which have invaded locally, and even in metastatic foci, we have now observed basement membrane production in a variety of human malignant epithelial neoplasms.

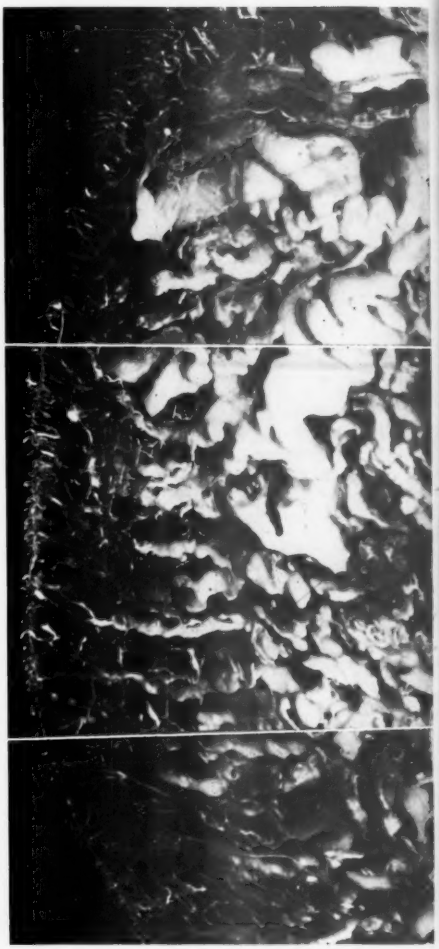

FIGURE 2 (Zinser)

We find Zinser's photomicrographs of stereoscopic views of the capillary system of cervical epithelium very impressive. These changes may be correlated with our demonstration, with PAS stains, of delicate capillary strands having apparently been pulled up into the squamous epithelium of carcinoma *in situ*. It might be questioned whether this is a manifestation of the downward movement of the atypical squamous epithelium or the upward growth of capillaries.